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14565

Development of Thiamine Deficiency in the Cat on a Diet of Raw Fish.

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Foxes fed a nutritionally adequate diet mixed with pieces of raw carp develop signs of thiamine deficiency within 20 days (Green, Carlson, and Evans[1]) due to the presence of an anti-thiamine factor present in the tissues. The enzymatic nature of this factor is indicated by its destruction on cooking, a possibility greatly strengthened by the success of Sealock, Livermore, and Evans[2] in extracting from carp tissues a dried powder with protein characteristics having anti-thiamine activity.

Previous studies in this laboratory (Everett[3]) on cats fed a diet of autoclaved canned rabbit meat supplemented with B vitamins other than thiamine led us to investigate the effects of raw fish diet in this species. This was done by feeding cats a diet consisting exclusively of either raw carp, herring, catfish, butter-fish, or spots, all purchased in the local markets. The animals were weighed weekly and a close watch was kept on their behavior. Carp and herring alone of the fish tried produced thiamine deficiency symptoms within the expected time. The results of the raw carp diet are summarized in Table I. On such a diet the cats developed all the signs of thiamine deficiency, including convulsions, within 23 to 40 days, with the exceptions noted later. The weight curve of cat 5 in Fig. 1 is typical of those animals succumbing to the diet. Several animals given subcutaneous injections of thiamine (as shown in Table I) when signs of deficiency were well advanced recovered without exception. Cat 7 in Fig. 1 is such an example. The deficiency symptoms developed as a result of eating raw carp are identical in every respect with those seen on the carefully controlled thiamine-free diet of autoclaved canned rabbit meat described by Everett[3] and in our opinion the two conditions are identical. The first sign of deficiency is anorexia with weight loss, followed within 10 to 20 days by the appearance of a staggering gait due to ataxia of the hindlegs, increasing weakness, circus movements, head ventro-

1 Green, R. G., Carlson, W. E., and Evans, C. A., *J. Nutrition*, 1942, **23**, 165.

2 Sealock, R. R., Livermore, A. H., and Evans, C. A., *J. Am. Chem. Soc.*, 1943, **65**, 935.

3 Everett, G. M., *Am. J. Physiol.*, 1944, **141**, 439.

TABLE I.
Results of Feeding Cats on Exclusive Diet of Raw Carp.

Cat No.	Days on diet	Initial wt, kilo	Minimal wt, kilo	% change in body wt	Remarks
1	38	2.8	1.85	34	Convulsive, killed
2	32	3.0	2.18	27	" died
3	66	3.1	3.1	0	No signs of B_1 deficiency
4	34	2.25	1.95	13	Convulsive, recovery on B_1 inj.
5	36	3.1	2.50	19	" killed
6	29	2.2	1.75	20	" "
7	35	2.3	1.6	31	" recovery on B_1 inj.
8	40	3.7	2.9	21	" killed
9	28	3.87	2.6	33	" died
10	23	2.78	1.7	39	" killed
11	37	2.50	1.85	26	" "
12	37	2.67	1.6	40	" "
13	31	3.05	2.1	31	" "
14	36	2.90	2.07	28	" "
15	77	4.02	2.91	25	" "

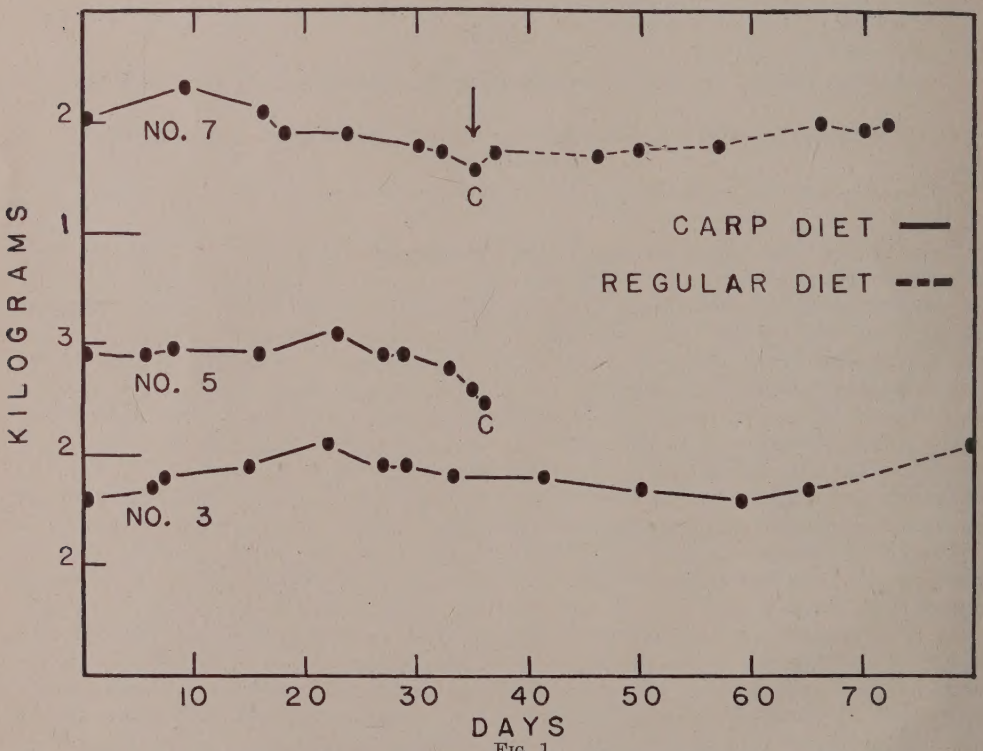

FIG. 1.
Weight changes in cats fed a diet of raw carp. ↓—thiamine injection; C—convulsions.

flexion, loss of righting reactions, convulsions, prostration, and death. Unless given thiamine the cats invariably died within 2 to 3 days after developing locomotor difficulties. Two cats were unusually resistant to the diet and remained alive 66 and 77 days, respectively, on raw carp. One of these was Cat 3. This animal was returned to the regular stock diet on the 66th day, having failed to develop any signs of thiamine deficiency. Cat 15, the

other one in this group, became convulsive on the 77th day of the carp diet and was killed. The behavior of these 2 animals suggests the possibility that in some cats the anti-thiamine factor is either partially destroyed by intestinal action or the animals for some time obtain thiamine from a source beyond the reach of the anti-thiamine factor, either by coprophagy or refection of the sort known to occur in the rat.[4,5]

Six cats fed an exclusive diet of salt water herring also developed thiamine deficiency in exactly the same manner as those on carp. However, the cats fed perch, cat-fish, butterfish, or spots either maintained their usual weights or else gained during the course of the experiment. The feeding was discontinued after there was no longer any reasonable expectancy that thiamine deficiency would develop. These findings support the conclusion of Deutsch and Hasler[6] that the anti-thiamine factor is not found in all fishes.

Summary. Cats fed an exclusive diet of raw carp or raw herring develop all the signs of thiamine deficiency characteristic for this species.

[4] Guerrant, N. B., and Dutcher, R. H., *J. Biol. Chem.*, 1935, **110**, 233.

[5] Kelly, E., and Parsons, H. T., *J. Nutrition*, 1937, **13**, 453.

[6] Deutsch, H. F., and Hasler, A. D., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 63.

14566

Influence of Pantothenic Acid Deficiency on Resistance of Mice to Experimental Poliomyelitis.*

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The relation of the nutritional status of a host to resistance in infectious diseases has received more detailed attention in recent years, largely because advances in the science of nutrition have made possible the study of the effects of an uncomplicated deficiency. Because of the intimate relation of the vitamins of the B-complex to cellular metabolism we have been investigating the influence of different levels of these vitamins on the incidence of experimental poliomyelitis. We[1] have previously reported the effect of thiamine deficiency on the resistance of Swiss mice to both Lansing strain poliomyelitis virus and Theiler's encephalomyelitis virus. In this paper similar studies are presented for pantothenic acid.

Experimental. Swiss mice (21-23 days old), bred in our laboratory, were used in all experiments and were kept in individual screen bottom cages supplied with a water bottle and an ointment jar containing the ration. Split litter technic, with consideration for sex and weight of mice, was employed. The synthetic pantothenic acid-low basal ration 503, employed in these studies, was composed of the following parts per 100: sucrose 73, vitamin-free casein 18, salt mixture 4,[2] and corn oil 5. The vitamin supplements per 100 grams of diet were: nicotinic acid 0.5 mg, riboflavin 0.3 mg, pyridoxine 0.3 mg, inositol 100 mg, para amino benzoic acid 100 mg, biotin (crude conc.) 0.01 mg, and choline 300 mg. This diet was supplemented with 0.05-0.1

* These studies were aided by a grant from the National Foundation for Infantile Paralysis, Incorporated.

We are indebted to Merck and Co., Rahway, N.J., for the synthetic vitamins and to Abbott Laboratories, North Chicago, Ill., for halibut liver oil.

[1] Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Infect. Dis.*, 1944, **74**, 41.

[2] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

TABLE I.
Influence of the Level of Pantothenate Intake on Resistance of Mice to Poliomyelitis (Experiment 35).

No. of days after inoculation	No. of days on ration	% of mice paralyzed					
		Theilers G-D VII (0.1% conc.)			Lansing (5% conc.)		
		deficient (25)*	optimum (28)	high (18)	deficient (26)	optimum (25)	high (20)
6	20	8	18	6	27	32	40
7	20	32	32	33	35	40	45
8	20	40	57	44	38	40	55
9	20	48	86	72	42	48	60
10	20	48	93	88	46	52	60
11	20	68	96	94	46	52	65
14	20	72	100	94	50	60	70
28	20	72	100	94	54	64	70
		Theilers G-D VII (1%)					
		(10)	(18)	(10)			
7	36	0	6	20			
8	36	0	11	30			
9	36	9	22	40			
10	36	9	33	40			
11	36	9	50	50			
14	36	20	66	60			
28	36	30	72	70			

* Numerals in parentheses indicate number of mice inoculated.

ml of halibut liver oil per mouse per week, given *per os* twice weekly at the time of weighing.

On this diet, without the pantothenic acid, young mice gained slowly for about 2 weeks, then exhibited a definite weight plateau followed by gradual weight loss. The deficiency signs were a gradual loss of fur, apathy, and occasional dermatitis around the nose. Mice gained weight rapidly and appeared normal in every respect when fed ration 503 plus 2.5 mg calcium pantothenate per 100 g of diet (optimum level) or 503 plus 25 mg calcium pantothenate per 100 g of diet (high level).

The viruses employed, Lansing strain poliomyelitis and Theiler's encephalomyelitis (GD VII strain), were given by the intracerebral route using an inoculum of 0.03 ml, and in the concentrations indicated. Uninoculated control mice were maintained on the same rations to determine the effect of the diets alone, and to decide the optimum time for virus inoculation. The mice were observed twice daily after inoculation for signs of flaccid paralysis; the experiments were terminated 28 days after administration of the virus.

In Experiment 35, Table I, mice were inoculated with virus suspensions 20 days after being placed on the synthetic ration. The concentration of Theiler's virus was in this series 0.1% and Lansing 5%. The most striking differences in paralysis were observed on the tenth day following administration of virus. The results at this time in the deficient, optimum, and high diets were 12 of 25 mice (48%), 26 of 28 mice (93%), and 16 of 18 mice (88%) respectively. At the end of 28 days, 18 of 25 mice (72%) on the deficient ration, had developed Theiler's encephalomyelitis, while, of 28 mice on the optimum ration, 28 (100%) had developed the infection. The mice fed the diet high in calcium pantothenate showed a paralytic incidence of 94%. In the same experiment with Lansing strain poliomyelitis virus the results were as follows: in the deficient group 14 of 26 mice (54%) were paralyzed, in the optimum group 16 of 25 mice (64%) developed the disease, and 14 of 20 mice (70%) fed the ration containing a high level of calcium pantothenate were typically paralyzed.

The results of this experiment suggested that pantothenic acid played some role in the incidence of infection of mice to Theiler's virus, but that the vitamin had little effect when Lansing virus was employed.

In order to ascertain the effect of a prolonged pantothenic acid deficiency the remain-

TABLE II.
Influence of the Level of Pantothenate Intake on Resistance of Mice to Poliomyelitis (Experiment 37).†

No. of days after inoculation	% of mice paralyzed			
	Theilers G-D VII (10% conc.)		Lansing (5% conc.)	
	deficient (29)*	optimum (35)	deficient (28)	optimum (34)
6	3	6	0	9
7	7	26	7	9
8	31	49	14	12
9	48	69	18	15
10	52	80	25	15
11	59	91	36	18
14	59	100	43	24
19	59	100	50	38
28	59	100	50	41

* See footnote Table I.
† All mice inoculated 27 days after being placed on synthetic ration.

ing control mice from Experiment 35 were inoculated with a 1% suspension of Theiler's virus 36 days after being placed on the synthetic ration. Although the number of mice was small, the data supported our previous experience (Table I). Of 10 pantothenate-deficient mice only 3 (30%) were paralyzed at the end of 28 days, of 18 mice fed a diet optimum in pantothenic acid 13 (72%) developed the disease, while 7 of 10 mice (70%) on a high diet showed signs of infection.

In Experiment 37 (Table II), essentially a repetition of Experiment 35, the virus was administered 27 days after the basal ration was given. Both viruses were again used, the concentration of Theiler's virus being in this instance 10% and Lansing 5%. The results again indicated that the mice fed a ration devoid of calcium pantothenate and inoculated with Theiler's virus suffered a lower incidence of infection than those mice getting a diet optimum in pantothenate requirements. Seventeen of 29 mice (59%) on the deficient ration developed paralysis, while 35 of 35 mice (100%) fed the diet optimum in pantothenate showed signs of the disease. It should be noted that the virus suspension employed was 100 times and 10 times more concentrated respectively than those used in the first and second parts of the previous experiment. There was no significant difference in the incidence of paralysis when the Lansing virus was administered: 14 of 28 deficient mice (50%) were paralyzed, and 14 of 34 mice (41%) on the optimum ration developed experimental poliomyelitis.

Discussion. One might assume that an increased resistance to experimental virus infections would occur in nutritionally deficient animals on the rationale that if the virus is to be considered an intracellular parasite it would be entirely dependent on the cellular metabolism of the host. Any disturbance in the normal metabolism might therefore affect the virus contained in these cells. However, in the present experiments 2 viruses were employed each influencing nerve cells of the central nervous system. Yet the deficiency in pantothenate definitely altered the incidence of Theiler's infection and had little or no influence on the course of the disease following inoculation with Lansing virus. The possibility exists that in this deficiency there is a depletion of some metabolite necessary for the propagation of Theiler's virus but not for Lansing virus, or that there is an accumulation of a metabolite inhibitory to the former virus but not to the latter. Further studies are necessary in order to elucidate this aspect of the problem.

Conclusions. In 2 series involving a total of 348 Swiss mice, those fed a synthetic ration deficient only in calcium pantothenate exhibited a definite increased resistance to Theiler's encephalomyelitis, but little or none to the Lansing strain of poliomyelitis.

14567

Dietary Fat with Reference to the Spontaneous Appearance and Induction of Leukemia in Mice.*

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Diets high in fat have been reported to increase the rate of formation of certain types of induced tumors.[1,2,3,4] The total number of induced tumors was also increased when threshold amounts of carcinogen were used.[4,5] The induction of some types of tumors has been found, however, to be unaffected by the amount of fat in the diet.[2,4,6] The external presence of fat at the site of percutaneous application of carcinogen was considered to be at least partially responsible for the accelerating effect in the induction of skin tumors.[3,7,8,9] For spontaneous mammary cancer in virgin Dba mice, the total tumor incidence was greater and the time of appearance earlier if the animals were fed a diet containing 12 rather than 3% fat;[4] food intake of animals in both groups was approximately isocaloric, although the mice were fed *ad libitum*. Lavik and Baumann[9] positively correlated caloric intake with number of induced skin tumors when animals were fed relatively high and low fat diets. The high fat animals consumed 12 to 30% more calories and developed more tumors than mice on the low fat diet. Recently dietary coconut oil has been found to exert an inhibiting effect on the genesis of induced hepatic tumors of rats.[10]

In the present experiments the effects of diets relatively high (32%) and low (3%) in fat were tested on (1) the appearance of spontaneous leukemia in the high leukemia F strain[11] and (2) the carcinogenic induction of leukemia in strain Dba. The diets used were modified from those described by Tannenbaum.[4] Each diet contained 250 parts of Purina fox meal, 100 parts skimmed milk powder, 30 parts brewers' yeast, and 8 parts of cod liver oil. The high fat diet contained in addition 180 parts of hydrogenated cottonseed oil,† and the low fat diet 405 parts, or an equicaloric amount, of cornstarch. Animals were fed *ad libitum*.

Strain F mice were placed on these diets shortly after weaning age. Litter mates were divided so that half were fed each of the diets. Growth rate was more rapid in mice on the high fat diet; progressively their caloric consumption became significantly greater. At one year of age the average weight of the high fat animals was 23 g as compared with an average of 18.4 g in the low fat group. Table I presents data indicating a slightly earlier appearance of leukemia in the high fat group, although the total incidence of leukemia has been approximately the same for both groups.

It is unlikely that the difference in age of onset of spontaneous leukemia in the two groups, if significant, can be attributed to the variable of fat content of the two diets. Dif-

* This investigation was aided by grants from The Jane Coffin Childs Memorial Fund for Medical Research and the Cancer Fund of the Graduate School of the University of Minnesota.

1 Baumann, C. A., and Rusch, H. P., *Am. J. Cancer*, 1939, **35**, 213.

2 Baumann, C. A., Jacobi, H. P., and Rusch, H. P., *Am. J. Hyg.*, 1939, **30**, 1.

3 Jacobi, H. P., and Baumann, C. A., *Am. J. Cancer*, 1940, **39**, 338.

4 Tannenbaum, A., *Cancer Research*, 1942, **2**, 468.

5 Lavik, P. S., and Baumann, C. A., *Cancer Research*, 1941, **1**, 181.

6 Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A., *Cancer Research*, 1941, **1**, 699.

7 Morton, J., and Mider, G. B., *Pub. Health Rep.*, 1940, **55**, 670.

8 Rusch, H. P., Baumann, C. A., and Kline, B. E., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 508.

9 Lavik, P. S., and Baumann, C. A., *Cancer Research*, 1943, **3**, 749.

10 Miller, J. A., Kline, B. E., Rusch, H. P., and Baumann, C. A., *Cancer Research*, 1944, **4**, 153.

11 Kirschbaum, A., and Strong, L. C., *Am. J. Cancer*, 1939, **37**, 400.

† The hydrogenated cottonseed oil (Kremit) was kindly supplied by the Armour Company.

TABLE I.
Age Incidence of Leukemia in Strain F Mice on High and Low Fat Diets.

Age in days	High fat (48 mice)			Low fat (48 mice)		
	Leukemic	Non-leukemic	Living	Leukemic	Non-leukemic	Living
0-100						
101-200						
201-300	6			2	1	
301-400	6			6	1	
401-500	9	2	3	9	1	2
501-600	6	3	8	12	1	12
601-700		2	3		1	
Totals	27	7	14	29	5	14

ferences in caloric intake and growth of the two experimental groups could account for the results obtained.

In order to test the effect of dietary fat on the carcinogenic induction of leukemia, methylcholanthrene (0.5% solution in benzene) was applied percutaneously twice weekly to 49 strain Dba mice (subline 12) fed the high fat diet, and to 48 mice of the same strain and subline which were fed the low fat diet Mice of this subline are susceptible to the induction of leukemia with this treatment.[12] Administration of carcinogen was begun when mice were 8 weeks of age and had been fed their respective diets for 4 weeks. Of those mice on the high fat diet, 16 of 22 males and 14 of 27 virgin females developed leukemia after average latent periods of 143 and 135 days respectively. Of those on the low fat diet, 21 of 33 males and 12 of 15 virgin females were leukemic after average induction periods of 116 and 113 days. Food intake was approximately isocaloric and there was no appreciable difference in the weights of the high and low fat animals. Frequency distribution curves plotted from induction periods of individual mice on the two diets indicate that the delay in onset of carcinogen-induced leukemia of mice on the high-fat diet was statistically significant.

It would seem that although the leukemia incidence was the same for both dietary groups, the induction of the disease was significantly delayed when the diet contained a relatively high per cent of fat. The oil on the fur of the high fat animals might have retarded absorption of carcinogen, thus prolonging the induction period.

[12] Kirschbaum, A., Lawrason, F. D., Kaplan, H. S., and Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1944, in press.

14568 P

Prevention of Neuroma Formation by Encasement of the Severed Nerve End in Rigid Tubes.

EDGAR J. POTH AND E. BRAVO-FERNANDEZ. (Introduced by Chauncey D. Leake.)

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Neuromata characteristically form at the proximal end of sectioned peripheral nerves. These tumors are frequently painful and represent a most troublesome problem in the handling of amputation stumps. The methods recommended in the Neurosurgical section of the Military Surgical Manual[1] to prevent such neuroma formation are: (a) the alcohol injection of the stump as suggested by Huber and

[1] National Research Council: Military Surgical Manual, Vol. 6, W. B. Saunders Co., Phila., 1943.

Lewis,[2] (b) the excision of a wedge of the nerve end and suture of the flaps (Corner[3]), and (c) a combination of the two procedures as proposed by Stookey.[4] Kirk[5] described a procedure in which the end of the nerve is cauterized with the high frequency current. Boldrey[6] reported an experimental method for overcoming neuroma formation by burying the stump of the nerve in bone. While these procedures frequently reduce the size to which neuromata grow, they have not been universally effective in preventing the development of painful amputation stumps.

In an attempt to completely inhibit neuroma formation, by controlling the growth of the nerve fibers and connective tissue, the cut ends of the sciatic nerve of the dog were placed in snugly fitting, rigid tubes of various materials: silver, cellophane, vitalium, or glass.

This experimental procedure has been carried out on 24 animals. After as long as 102 days the fibers of the sciatic nerve encased in a snugly fitting glass tube are straight and orderly and there is no tendency towards neuroma formation. All control experiments in which a portion of nerve was resected showed neuroma formation.

Conclusion. When the cut end of the sciatic nerve of the dog is supported by a closely fitting, rigid tube, there is no tendency to disorderly overgrowth of the nerve fibers and connective tissue characteristic of neuroma formation.

2 Huber, G. C., and Lewis, D., *Arch. Surg.*, 1920, **1**, 85.

3 Corner, E. M., *Proc. Roy. Soc. Med.*, **11**, 7, April 9, 1918.

4 Stookey, B., *Surgical and Mechanical Treatment of Peripheral Nerves*, p. 461, chap. 21, W. B. Saunders Co., Phila., 1922.

5 Kirk, N. T., Amputations: Dean Lewis, *Practice of Surgery*, Vol. III, chap. 10, 1943.

6 Boldrey, E., *Ann. Surg.*, 1943, **118**, 1056.

14569

In vitro Action of Penicillin Alone, and in Combination with Sulfathiazole, on *Brucella* Organisms.

TSUN T'UNG. (Introduced by Martin Frobisher, Jr.)

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Information concerning the antibacterial effect of penicillin on gram-negative organisms is meager and it is a general belief that most gram-negative bacilli, including *Brucella*,[1] are relatively resistant to penicillin. This has led to possibly undue pessimism with regard to the therapeutic use of available chemotherapy in brucellosis. The present communication presents *in vitro* studies of the action of penicillin alone, and in combination with sulfathiazole, on a number of strains of *Brucella* organisms.

Materials and Methods. Penicillin was prepared from a Fleming strain of *Pen. notatum* cultivated on modified Czapek-Dok medium[2] to which 1% of casamino acid was added. The potency of the crude penicillin on the 7th day when tested by the serial dilution method was around 1-640 against a test strain of *Staphylococcus aureus* H (3R9674). The pH of the culture fluid on the 7th day was about 7.5. Extraction with amyl acetate and buffer was carried out according to the procedure of Abraham *et al.*[3] Extraction was repeated once to concentrate the penicillin. The extracted material was shaken with 2% animal charcoal

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **11**, 177.

2 Hobby, Gladys L., and Meyer, Karl, PROC. SOC. EXP. BIOL. AND MED., 1943, **50**, 227.

3 Abraham, E. P., and Chain, E., *Brit. J. Exp. Path.*, 1942, **23**, 103.

TABLE I.
Bacteriostatic Action of Penicillin on *Brucella* Organisms.

Organisms	Dilution of penicillin*								
	20	40	80	160	320	640	1280	2560	5120
Suis									
Zin	—	—	—	—	—	—	—	3	4
Gilley	—	—	—	—	—	—	—	4	
Harrison	—	—	—	—	—	—	1	4	
Svic	—	—	—	—	—	—	2	4	
Stock	1	4							
Abortus									
Green	—	—	—	—	—	—	—	—	4
No. 9	—	—	—	—	—	—	—	4	
Stock	—	—	—	—	—	—	4		
No. 7	—	2	4						
Croni	—	4							
Melitensis									
No. 3	—	—	—	—	—	—	—	4	
Stock	1	3	4						
Gilly	1	4							
Bent.	2	4							
No. 1	3	4							

* A dilution of 1/320 indicates a concentration of 1 Oxford unit per cc.
Minus sign indicates no growth; numerals indicate the degree of turbidity of growth.

and filtered through Seitz pads. The filtrate has a slight amber color. The partially-purified penicillin, when tested by the cup-plate and serial dilution methods,[4] was found to contain 320 Oxford units per ml. The penicillin was then put into celluloid tubes and preserved in the low-temperature (–76°C) cabinet until used.[5]

Sodium sulfathiazole, dissolved in physiological saline solution in a concentration of 50 mg per ml, was sterilized in boiling water for 20 minutes. The solution was further diluted with broth so that 0.1 ml added to 0.9 ml of medium containing various amounts of penicillin, gave a concentration of 5 mg % of sodium sulfathiazole. For sulfathiazole-susceptible strains the amount of sulfathiazole used in conjunction with penicillin was reduced to that which, alone, produced only slight bacteriostasis.

Meat infusion broth (pH 7.4) containing 0.25% dextrose was used for all *Brucella* cultures. Penicillin was diluted with this broth. In some tests one-tenth ml of the penicillin-containing medium was replaced with the same amount of sulfathiazole-containing broth so that the volume of the medium was maintained at one ml in all test cultures in serological tubes.

The inoculum in all tests consisted of one drop of a 1-10 dilution of a 24-hour broth culture, introduced into the medium from a syringe with a 26-gauge needle. Each drop contained approximately 100 million organisms. This heavy inoculum was necessary to obtain a clear-cut and uniform result in a comparatively short time without resorting to cultivation in an atmosphere containing added CO_2

Cultures were incubated at 37°C. Since a luxuriant growth could not be obtained in the sulfathiazole-containing medium within 48 hours under the conditions of the tests described, readings were taken after 72 hours' incubation. The contents of tubes showing no visible growth were plated out on tryptose agar to detect possible slight growth or bacteriostatic action.

All of the strains used had been preserved for a year or more at –76°C. A majority of them were placed at this temperature soon after they were isolated. The stock cultures, *i.e.*, those which have been kept and trans-

[4] Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1944, **47**, 43.

[5] Tung, T., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 103.

TABLE II.
Action of Penicillin in Combination with Sodium Sulfathiazole on *Brucella* Organisms.

Organisms	Dilution of penicillin										Sod. sulfathiazole alone mg %		
	20	40	80	160	320	640	1280	2560	5120	10240	5	1	0.1
Suis													
Zin	—	—	—	—	—	—	—	1	3		—	3	
Gilly	—	—	—	—	—	—	—	—	1	4	4		
Harrison	—	—	—	—	—	—	—	4			4		
Svic	—	—	—	—	—	—	—	2			3		
Stock	—	—	2	2							—	—	2
Abortus													
Greene	—	—	—	—	—	—	—	—	1	3	3		
No. 9	—	—	—	—	—	—	—	—	1	3	3		
Stock	—	—	—	—	—	—	—	2	2	2	—	—	2
No. 7	—	—	—	4							4		
Croni	—	—	1	2	3						3		
Melitensis													
No. 3	—	—	—	—	—	—	—	—	1	2	2		
Stock	—	—	—	1	2						—	—	2
Gilly	—	3									3		
Bent	—	3									3		
No. 1	1	4									4		

ferred for many generations on ordinary infusion agar, though of low virulence still possess their distinctive cultural characteristics.

Results. The bacteriostatic action of penicillin on *Brucella* organisms is shown in Table I. It can be seen that the 15 strains tested fall into two groups according to their relative susceptibility to penicillin. Eight strains were susceptible to penicillin at a concentration of about 0.1-0.5 unit whereas even 10 units could not inhibit the growth of the other strains. It seems that there are strain, instead of species, differences between the susceptibilities of *Brucella* organisms to penicillin. The data suggest that the *suis* variety is more penicillin-susceptible than are the melitensis strains, but the number of strains in each group is too small to permit definite conclusions on this point.

Penicillin in the greater concentrations exerted a definitely bactericidal effect on some of the strains. The plating test with the inoculated tubes in which no growth was visible showed that a bactericidal action was exerted by concentrations of penicillin one or two dilution intervals below those producing only a bacteriostasis.

Since, in order to be effective on some strains of *Brucella,* concentrations of penicillin are required which are attainable but not maintainable under clinical conditions, it was regarded as worth while to try the combination of a representative sulfonamide drug with penicillin[6] in the hope of producing a synergistic action. It was hoped that certain strains could thereby be rendered vulnerable to lower concentrations of penicillin. Sodium sulfathiazole was chosen for this experiment, being apparently the most effective among sulfonamides against organisms of the *Brucella* group.[7,8] The action of penicillin in combination with a small amount of sulfathiazole is presented in Table II. It can be seen that penicillin, combined with sulfathiazole, exerts a considerably greater antibacterial action than penicillin alone. This synergistic action is more noticeable on certain strains.

These experiments, in which large inocula were used, incidentally demonstrated great differences between susceptibilities of *Brucella* organisms to sulfathiazole. While the stock strains and one of the *suis* strains proved extremely susceptible to sulfathiazole, most of the recently isolated cultures were so resistant to this drug that a concentration of

6 Ungar, J., *Nature,* 1943, **152**, 254.

7 Kempner, W., Wise, B., and Schlayer, C., *Am. J. Med. Sci.,* 1940, **200**, 484.

8 Wise, Bowman, *J. Pharm. and Exp. Ther.,* 1942, **76**, 156.

even 5.0 mg % was not appreciably inhibitory to them. There seems to be no parallelism between the susceptibilities of *Brucella* organisms to the two therapeutic agents tested.

It is beyond the scope of this experiment to estimate how much of the antibacterial action of the penicillin preparation used was due to penicillin itself and how much to the so-called penatin.[9,10,11] However, it may be pointed out that the penicillin was harvested at pH 7.5 and that it was extracted with an organic solvent. Moreover, neither the presence nor absence of a small amount of dextrose in the medium for the tests appreciably affected the antibacterial action of this material. These facts suggest that it was the penicillin rather than penatin that was chiefly responsible for the antibacterial action.

Conclusion. Penicillin exerted a considerable antibacterial action on 8 out of 15 strains of *Brucella in vitro*. This action was enhanced by the combination of penicillin with a small amount of sodium sulfathiazole.

9 Kocholaty, Walter, *J. Bact.*, 1942, **44**, 469.

10 Kocholaty, Walter, *Arch. Biochemistry*, 1943, **2**, 73.

11 Kocholaty, Walter, *Science*, 1943, **97**, 186.

14570

Absence of Liver Damage in Chicks Hypoprothrombinemic Due to Vitamin K Deficiency or Ingestion of 3,3′-methylenebis (4-hydroxycoumarin)*

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It is generally assumed that the hypoprothrombinemia resulting from vitamin K deficiency is due to the inability of the liver to synthesize prothrombin in the absence of an adequate amount of vitamin K. In chicks reared on an artificial vitamin K-free diet which produced clinical signs of the deficiency disease within 12 to 14 days, one group of investigators[1] observed fatty degeneration and necrosis in the liver and suggested that the absence of vitamin K may cause liver damage which may in turn be responsible for the failure of prothrombin formation. However, patients suffering from hypoprothrombinemia which can be corrected by vitamin K do not as a rule show an abnormal response in the usual tests of liver function.

The first description of the hemorrhagic disease in cattle caused by the feeding of spoiled sweet clover hay also mentioned liver damage accompanying the hypoprothrombinemia.[2] However, investigations of the sweet clover disease carried out since the isolation and synthesis of the causative agent, 3,3′-methylenebis (4-hydroxycoumarin), commonly referred to as dicumarol, have left undecided the question of the association of liver damage with this form of hypoprothrombinemia.[3,4]

The modes of action of vitamin K and dicumarol in the body remain unexplained; but in view of the possibility that their action might be expressed in morphologic as well as functional alterations in the liver, it seemed desirable to undertake a more detailed exam-

* Aided by a grant from the Josiah Macy, Jr., Foundation. We are indebted to Dr. K. P. Link and to Eli Lilly and Co. for supplying the dicumarol; and to Hoffman-LaRoche, Inc., for supplying the *d,l*-alpha-tocopherol acetate and Synkavite (tetra sodium salt of 2-methyl-1,4-naphthohydroquinone diphosphoric acid) used in these experiments.

1 Hepding, L., and Moll, T., *E. Merck's Jahresbericht*, 1939, **53**, 5.

2 Schofield, F. W., *Can. Vet. Rec.*, 1922, **3**, 74.

3 Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 513.

4 Overman, R. S., Field, J. B., Baumann, C. A., and Link, K. P., *J. Nutrition*, 1942, **23**, 589.

TABLE I.
Chicks Fed Commercial Diet.

No. of animals	Age, days	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
7	36-63	100	0	Fasted 18-24 hr.
5	37-62	100	0	Fed

TABLE II.
Chicks Fed Commercial Diet. Dicumarol Given by Mouth.

Animal	Age, da.	Clotting power, % normal	Liver fat	Dicumarol mg/da./g.b.w.	Dicumarol Days treated	Treatment prior to sacrifice
573	54	9.1	0	0.25	3*	Fasted 18 hr.
574	63	10	0	0.35	5*	" 25 "
575	56	5.2	0	0.25	5*	" 24 "
583	49	16	0	0.23	2*	" 27 "
2378	58	1	0	1.06	1†	" 24 "
2459	58	2	0	1.06	1†	" 24 "

* Killed 24 hr. after last dose of dicumarol.
† Killed 48 hr. after receiving dicumarol.

ination of the liver in a series of hypoprothrombinemic animals. The results of a study of 40 chicks subjected to various experimental procedures have shown that on a proper dietary regimen marked hypoprothrombinemia can be produced in these animals with no morphologic evidence of liver damage demonstrable by the methods employed.

Methods. White leghorn chicks were used. The animals weighed from 300 to 500 g at the time of the prothrombin determinations. Vitamin K deficiency was produced by feeding the following diet: alcohol-extracted casein 150 g, dried brewer's yeast (ether extracted) 100 g, salt mixture No. 3† 20 g, gelatin 80 g, gum arabic 50 g, sucrose 598 g, cystine 1 g, choline chloride 1 g, cod liver oil 50 g, *d,l*-alpha-tocopherol acetate 100 mg. Normal chicks were given a commercial chicken diet or the vitamin K-free diet plus 10 mg vitamin K substitute per kilogram. Dicumarol was fed as a powder or as tablets to chicks receiving the commercial diet. The clotting power of the plasma, $(K_n/K)100$, was determined as described by Dam and Glavind[5] (K_n = concentration of thromboplastin which clots plasma of normal chicks in 3 minutes under standard conditions; K = concentration of thromboplastin which clots the plasma to be tested in the same length of time.) For histologic studies tissues were fixed in Bouin's solution followed by Mallory-azan staining, and in formol-saline followed by staining of frozen sections with Sudan IV for fat. Tissues were also fixed and stained by the method of Regaud for demonstrating mitochondria.

Observations and Comments. Post-mortem examinations revealed no gross pathologic changes in the liver of any of the animals. Mitochondrial shape and abundance as well as other general histologic features of the livers from the experimental animals showed some variation, but the variations within the experimental groups did not distinguish the latter from the corresponding control groups.

The liver specimens were classified according to the abundance of fat estimated by inspection of sections stained with Sudan IV. The most fatty livers contained fat in practically every hepatic cell. In some livers the fat was more abundant around the central veins, while in others it was uniformly distributed or more abundant in the portal areas. Sudanofil fat was not present in the livers of chicks receiving the commercial diet (Tables I and II), but did occur in more than 50%

†$CaCO_3$ 400, $MgCO_3$ 20, NaCl 88, ferric citrate 32, $CuSO_4 \cdot 5H_2O$ 3, $MnSO_4 \cdot 4H_2O$ 6, di-iodotyrosine 0.005.

5 Dam, H., and Glavind, J., *Biochem. J.*, 1938, **32**, 1018.

TABLE III.
Chicks Fed Vitamin K-free Synthetic Diet.

Animal	Initial age, da.	On diet, da.	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
325	19	27	20	++++	Fed
326	19	41	16	+	”
327	19	16	11	++	”
551	29	29	2.5	0	Fasted 26 hr.
553	29	29	2	0	” 28 ”
554	29	27	14	—	” 26 ”
555	29	27	30	++	” 24 ”
2491	7	30	0	+	Fed
2493	7	29	4	0	”
2494	7	34	0	0	”
2498	7	21	<1	+	”
2560	7	16	—	++	Fasted 24 hr.
328	19	16	5	—	Fed*
552	29	31	3	0	Fasted 26 hr.†

*, † Killed 19 and 25 hours respectively after receiving 95 γ of 2-methyl-1,4-naphthohydroquinone. Prothrombin then 100%.

TABLE IV.
Chicks Fed Synthetic Diet with Added Vitamin K (Synkavite, 10 mg/kg).

Animal	Initial age, da.	On diet, da.	Clotting power, % normal	Liver fat	Treatment prior to sacrifice
330	19	41	100	++	Fasted 14 hr.
331	19	10	100	0	Fed
332	19	16	100	+++	”
333	19	16	100	0	”
334	19	41	100	+	Fasted 15 hr.
335	19	27	100	+	Fed
2455	7	58	100	+	”
2457	7	58	100	0	”

of the animals receiving the synthetic diet (Tables III and IV). However, neither the incidence nor the severity of the fatty change was increased by vitamin K deficiency. Furthermore, severe hypoprothrombinemia was present in animals whose livers contained no sudanofil fat (Table III). The observed fat accumulation in the liver thus appears unrelated to the intake of vitamin K, and probably should not be interpreted as a pathologic degeneration but rather as a metabolic variation in individual response to the synthetic diet. Dietary abnormalities other than the absence of vitamin K might explain the marked fatty degeneration and liver damage reported by Hepding and Moll.[1]

The incidence of fatty change in the liver was the same in chicks fasted as in those fed prior to sacrifice. This is in contrast to observations on the mouse liver,[6] in which fasting for 24 hours produces a marked increase in sudanofil fat.

Schofield[2] distinguishes between a hemorrhagic and an anemic type of the sweet clover disease. Both types are characterized by anemia and hypoprothrombinemia, but in the first type extensive subcutaneous and internal hemorrhages are a prominent feature; while in the second type hemorrhagic manifestations are limited to petechiæ and ecchymoses which may be present in any of the serous membranes. Marked pathologic changes including intralobular hemorrhage, cloudy swelling, and fatty degeneration were found in the livers of cattle dying with the hemorrhagic form of the disease. We are not in a position to decide on the validity of this distinction, but the hypoprothrombinemia produced in chicks by dicumarol feeding is comparable to that described in cattle by Schofield; although as previously mentioned no gross or microscopic

[6] Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Stoneburg, C. A., Oleson, M. C., and Whitehead, R., *J. Biol. Chem.*, 1941, **139**, 897.

abnormality was observed in the livers of these chicks.

Since the completion of these experiments, Topelberg and Honorato[7] have reported that a vitamin K-free diet increases the total extractable fat of the chick liver, and that addition of vitamin K to the diet reduces the value toward that found in chicks fed a standard commercial diet. They also observed that injection of choline in the amount of 10 γ per animal per day would depress the fat content of the livers of chicks fed the artificial K-free diet. Our diet supplied much more choline than 10 γ per animal per day.

[7] Topelberg, G. S., and Honorato, C. R., *Rev. Soc. Argentina Biol.*, 1943, **19**, 409.

Summary. 1. Hypoprothrombinemia was produced in chicks by feeding a vitamin K-free diet, or by adding dicumarol to a standard commercial diet. 2. The livers of these animals showed no gross or microscopic changes which would distinguish them from corresponding groups of control animals. 3. Fatty infiltration of the liver which occurred in some of the chicks was related to the synthetic diet employed; but was not related to the lack of vitamin K or choline, or to the degree of hypoprothrombinemia. 4. In chicks there can be produced severe hypoprothrombinemia unassociated with histologic evidence of liver damage demonstrable by the methods employed in this study.

14571 P

Influence of Body Movement on Shock Due to Repeated Hemorrhage.*

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In the present experiments bodily movement is shown to have a beneficial effect on the mortality from repeated hourly hemorrhages in the dog. In a recent publication[1] a similarly beneficial effect was observed after severe trauma to both hind legs in animals allowed to move about as compared with those which were kept immobile in the supine position.

The present experiments were carried out as previously described except that instead of assuming the immobile supine position the animals were allowed to move freely about between each hemorrhage. Blood was removed (10 cc per kilo of body weight per hour) by puncture of the femoral artery through the intact skin except that it was often necessary after the initial hemorrhage to expose the contracted vessel. For this local anesthesia was employed when necessary. Hematocrit determinations were made from a portion of the removed blood. Twenty-five experiments were performed in all as described in Table I, of which 7 were controls. Of the 18 experiments permitted voluntary body movement, 6 were allowed nothing by mouth, 6 were allowed water *ad libitum*, and 6 were given a solution containing 5% hydrolyzed casein and pork pancreas (Amigen) and 5% glucose by gavage equal in amount to the blood which had been removed; it so happened that the volume of water voluntarily taken averaged the same as the amount given by gavage.

Experimental Findings. Hematocrit determinations revealed a drop in red cell volume of 20 to 30% below the initial value in all groups. The greatest drop occurred in those allowed water by mouth *ad libitum*, or given a solution of Amigen and water by gavage; of these 2, the drop was less rapid in the

* Aided by a grant from the Commonwealth Fund.

[1] Eversole, W. J., Kleinberg, W., Overman, R. R., Remington, J. W., and Swingle, W. W., *Am. J. Physiol.*, 1944, **140**, 490.

TABLE I.

	No. alive after onset							Avg	
Hours	0	1	2	3	4	5	6	Total bleeding (% of body wt)	Survival time (in hours)
1. Immobile in the supine position	7	7	7	5	0	0	0	3.9	3.1
2. Full body movement in cage									
a. Water withheld	6	6	6	6	6	0	0	4.9	4.2
b. Water *ad libitum*	6	6	6	6	6	1	0	5.0	4.5
c. Amigen and glucose by gavage	6	6	6	6	6	3	0	5.5	4.8

former. More striking were differences in the 2 groups given nothing by mouth in which there was a more pronounced drop in the hematocrit value in those immobile in the dorsal position, as compared with those allowed to move in the cage.

The most significant findings are those on the survival time and the amount bled as shown in Table I. The survival time in the controls kept immobile in the dorsal position averaged 3.1 hours. In similar experiments previously reported[2] the figure was 3.7 hours, which may have been due to a significant difference in technic, *i.e.*, ligation of the femoral artery was performed. In the present experiments the animals which were allowed to move in their cages survived from 4.2 and 4.5 hours, due to the fact that they were able to withstand at least one more hemorrhage than the controls. The beneficial effect of hydrolyzed protein and glucose by mouth is shown by the fact that two more animals survived the fifth bleeding in this group than in the next best group in which water only was ingested.

Summary. Voluntary body movement had a beneficial influence on the resistance of the dog to the fatal effects of repeated hemorrhage, as shown by a longer survival time, *i.e.*, the ability to sustain greater loss of blood, as compared with similar experiments carried out in the immobilized supine position.

2 Elman, R., and Lischer, C. E., *Ann. Surg.*, 1943, **118**, 225.

14572

Pathogenic Myxobacteria.

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During the summer of 1942 an epizootic occurred in a population of blueback-salmon fingerlings, *Oncorhynchus nerka* (Walbaum), which were being reared at the Fish and Wildlife Service hatchery at Leavenworth, Washington. The disease was recognized by Dr. F. F. Fish of the Fish and Wildlife Service as similar to, if not identical with, a bacterial disease occurring among warm-water fishes of the Mississippi Valley which was described by Davis.[1] Its occurrence as a disease of cold-water fishes has been described by Fish and Rucker.[2]

Davis gave an excellent description of the bacteria that appeared abundantly in lesions on the surfaces of infected fish, and proposed the name *Bacillus columnaris* for the species concerned. Of particular interest was his observation that when a little material was scraped from a lesion and placed in a drop of water on a slide, the bacteria present collected on the edges of infected tissues and scales to

1 Davis, H. S., *Bull. U. S. Bur. Fish.*, 1923, **38**, 261.

2 Fish, F. F., and Rucker, R. R., *Trans. Am. Fish. Soc.*, 1944, **73**, in press.

TABLE I.
Mortality of Blueback Salmon Fingerlings Exposed to Infection by Myxobacteria.

	Inoculated fish				Control
Temperature of Water in °C	16°	18°	20°	22°	22°
No. of fish	20	20	20	10	20
No. of fish dying	6	9	19	10	0
% mortality	30%	45%	95%	100%	0%

form short column-like masses. This striking movement occurred with material taken from lesions on blueback-salmon fingerlings and served to identify the infection.

Unfortunately, Davis was unsuccessful in his attempts to grow the bacteria on nutrient media. Though he obtained strong presumptive evidence of the causal relationship of *Bacillus columnaris* to the disease, failure to isolate the bacteria in pure culture rendered it impossible to demonstrate beyond question the cause of the disease. Apparently the investigation was carried no further, for no other references to this disease have been found in the literature.

The initial isolation of the bacteria, which occurred abundantly in the characteristic lesions of the disease in blueback salmon, was accomplished by serial dilution in a dilute fish-infusion medium. The bacteria failed to grow on media containing the usual concentrations of nutrients and agar but grew well at reduced concentrations. Subsequently, a medium containing 0.25% to 0.50% Bactotryptone and 0.5% to 0.9% agar adjusted to pH 7.3 was found to be very satisfactory for isolation and cultivation.

The organisms, isolated in pure culture, were recognized as myxobacteria. The vegetative cells were flexible, weakly refractive, Gram negative rods, usually measuring 0.5 to 0.7 by 4 to 8 microns. Spherical and oval microcysts varying in diameter from 0.7 to 1.2 microns were found in cultures on both liquid and solid media, and these increased in number with the age of the culture. On solid media the organisms showed the creeping motility characteristic of the myxobacteria.[3] Upon addition of a drop of water to the edge of a colony it was possible to observe the rapid flexing movements described by Bauer[4] in myxococci.

Colonies on tryptone agar appeared yellow, flat and irregular with an uneven edge in which swarming could be observed. After a few days, the colonies assumed a warty appearance with irregular elevated areas resembling immature fruiting bodies. The organisms liquefied gelatin rapidly but did not produce indol or reduce nitrates. Starch, cellulose and agar were not attacked. Sugars were not fermented, but glucose was oxidized. No growth was obtained on mineral, filtered glucose agar.

Transmission experiments under controlled conditions have shown the myxobacteria to be pathogenic to salmonid fishes. The mortality, however, depended upon the temperature at which the fish were held. Table I shows an experiment in which blueback-salmon fingerlings were placed in a dilute suspension of myxobacteria and then held in troughs. The temperature of the inflowing water was thermostatically controlled and held at the temperatures indicated. The experiment was terminated after 7 days.

It was usually possible to isolate the myxobacteria from the internal organs of infected fish as well as from lesions on the surface of the body. In one experiment typical myxobacteria were isolated in pure culture from the kidneys of 22 out of 25 fish showing the external lesions of myxobacterial infection.

A search for the source of the myxobacteria responsible for the epizootic at the Leavenworth hatchery led to the isolation of myxobacteria from well-developed surface lesions and from the internal organs of adult chinook and blueback salmon, steelhead trout, squawfish, whitefish, chubs, and suckers taken from the Columbia River. Apparently the organisms are well established in the Columbia River, and the available evidence indicates

[3] Stanier, R. Y., *J. Bact.*, 1940, **40**, 619.
[4] Bauer, E., *Arch. Protistenk.*, 1905, **5**, 92.

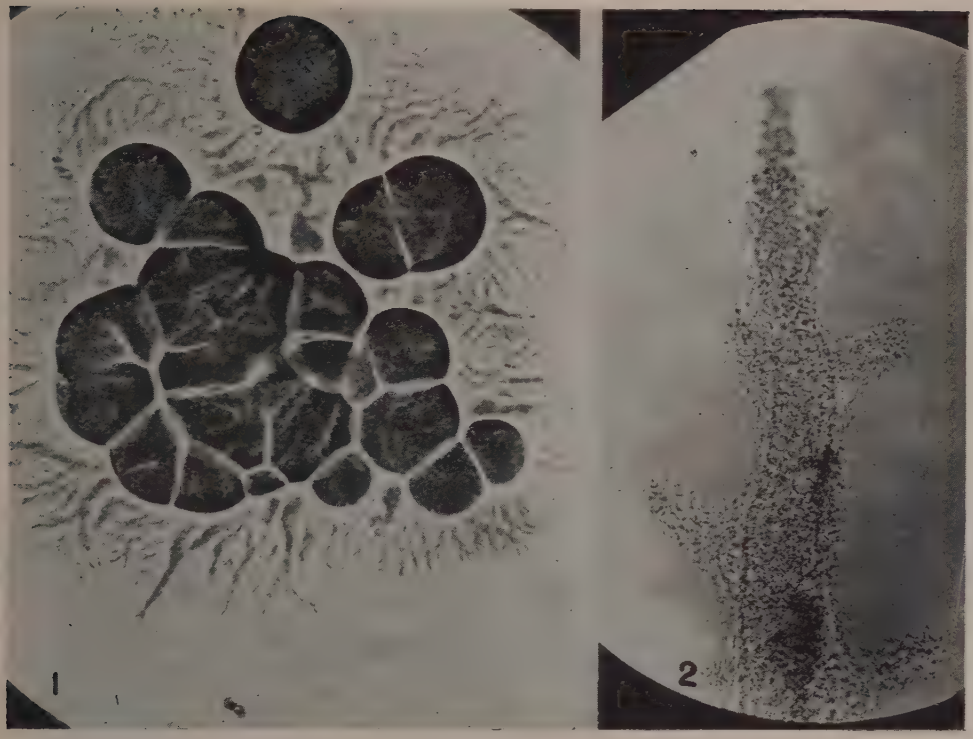

FIG. 1.
Fruiting bodies produced by *Chondrococcus columnaris* on tryptone agar. × 100.
FIG. 2.
Portion of a fruiting body produced by *Chondrococcus columnaris* in dilute tryptone broth. × 500.

that they may be responsible for a heavy mortality among native fish.

These cultures from Columbia River fishes were similar to those isolated at Leavenworth but exhibited some differences in growth on solid media. Of particular interest was the occurrence of definite fruiting bodies. These are illustrated in Fig. 1. Careful examination revealed the production of definite fruiting bodies on tryptone agar by all cultures listed above.

The phenomenon of column formation was studied using pure cultures. No column formation was observed when myxobacteria were suspended in water using ordinary wet mounts, though flexing and swinging movements were noted. In another attempt to obtain column formation, a series of cultures on sterile Brown slides was prepared. Young cultures of myxobacteria growing on tryptone agar were scraped off, placed in dilute tryptone broth on the Brown slides, and covered with sterile coverslips. In some cases sterilized scales from fish were introduced. Within 10 minutes short columns appeared at various points on the scales or at the edges of solid masses of bacteria. After 24 hours many long columns and branched structures were observed. One of these is illustrated in Fig. 2. It is apparent that these structures are fruiting bodies, since in a period of 7 to 10 days, the rod-shaped bacteria originally present were converted into spherical and oval microcysts.

The bacterial species here described clearly possesses the characteristics of the higher myxobacteria. On the basis of the shape of the microcysts the species should be assigned to the family Myxococcaceæ of the order

Myxobacteriales.[5] Following the key to the genera of the family Myxococcaceæ proposed by Stanier[5] the species should be placed in the genus *Chondrococcus,* because the fruiting bodies on agar are not deliquescent and are surrounded by a firm membrane. The species, therefore, may be properly named *Chondrococcus columnaris.* It should be pointed out, however, that the production of fruiting bodies in aqueous media is a new observation and is not considered in determining the systematic position of a myxobacterial species. It is possible that star formation, which was observed by Stapp and Bortels[6] and by Stanier[5] in liquid cultures of non-fruiting myxobacteria, is an analogous phenomenon.

We have begun an investigation of bacterial gill disease, a disease causing heavy loss of trout and salmon fingerlings. Cultures of myxobacteria have been consistently isolated from the gills of infected fish. These cultures, however, fail to produce organized fruiting bodies, and their systematic position has not yet been determined.

Summary. A myxobacterial species, *Chondrococcus columnaris,* has been isolated in pure culture and shown to produce a fatal disease in various fishes.

[5] Stanier, R. Y., *Bact. Rev.*, 1942, **6**, 143.

[6] Stapp, C., and Bortels, H., *Zent. Bakt. Parasitenk.*, 1934, II, **90**, 28.

14573

Relation of Sedimentation Rate to Amount of Precipitate Formed in Plasma by Type III Pneumococcus.*

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It has been known for many years that normal human sera contain considerable quantities of protein-polysaccharides.[1,2] According to Friedemann and Sutliff,[3] when sera from various pathologic states are inoculated with pneumococci, following an incubation period a voluminous white precipitate is obtained, while in normal sera only a slight cloudiness develops. This precipitate is not bacterial debris, but is caused by the production of unusually large quantities of acid which precipitate the serum proteins. The acids which are formed in normal sera by the organisms can be accounted for entirely by the free sugar which is fermented.[3] However, in abnormal sera growth continues at an undiminished rate after the free sugar has been completely utilized. Friedemann and Sutliff summarize their observations by stating that ". . . the phenomenon is due to the presence of abnormal quantities of a polysaccharide which supports rapid growth and which is readily fermented by the pneumococcus. It thus differs from the normally present polysaccharide, which is not apparently metabolized by the microörganism, since growth stops in normal sera when the free sugar is consumed."

These observations suggested the possibility that this abnormal polysaccharide which appeared in the blood might have some connection with the increased sedimentation rate of pathologic blood specimens. To investigate this, the following studies were carried out.

Methods and Results. Specimens of blood were obtained from a number of patients in the University Hospital, and normal blood samples were provided by members of the laboratory staff. Ten ml of blood were taken in the usual

* Submitted in partial fulfillment of the requirements for the degree of Doctor of Science in the University of Michigan.

[1] Rimington, C., *Biochem. J.*, 1929, **23**, 430.

[2] Hewitt, L. F., *Biochem. J.*, 1938, **32**, 1554.

[3] Friedemann, T. E., and Sutliff, W. D., *Science*, 1939, **90**, 335.

TABLE I.
Relation of Sedimentation Rate to Amount of Precipitate Formed in Plasma by the Growth of Type III Pneumococcus.

Clinical state	Precipitate formed in plasma inoculated with Type III pneumococcus*	Sedimentation rate in mm at the end of 1 hr
Normal	—	1
"	—	2
"	—	3
"	—	5
"	—	13
Ulcerative colitis	—	4
Essential hypertension	—	24
Neoplasm of stomach	+	8
Tuberculosis	+	30
"	++	3
Carcinoma of colon	++	75
Coronary infarction	++	75
Acute pharyngitis	+++	42
" rheumatic fever	+++	95
Pneumonitis (unknown etiology)	+++	112
Tuberculosis	++++	78
Lymphoblastoma	++++	80
Pneumococcus pneumonia	++++	110
Pneumonia (unknown etiology)	++++	113
Hypertension and upper resp. infection	++++	138

* Maximum precipitate following 48 to 96 hours of incubation.

manner from the median basilic vein and transferred promptly to a sterile tube containing 0.5 ml of 4.0% potassium oxalate; all specimens were kept at 4°C until used. Portions of each blood specimen were taken for the determination of the sedimentation rate within a few hours after they were obtained; a modified Westergren technic[4] was employed. The remainder of the blood specimens were centrifugated and the plasma removed.

A modification of the procedure Friedemann and Sutliff used with sera was applied to the specimens of plasma to determine the presence of non-specific polysaccharide. One ml of the clear plasma was transferred to a sterile serological tube and inoculated with 0.05 ml of a 12- to 18-hour 0.1% glucose broth culture of a strain of Type III pneumococcus. The tube was then incubated at 37°C and examined at frequent intervals.

The specimens of normal plasma became faintly cloudy (—); while plasma from the patients became either opalescent (+), almost opaque (++), opaque with a small amount of precipitate (+++), or contained a voluminous precipitate (++++). No attempts were made to determine the reducing sugar in the plasma to rule out any effect due to abnormal quantities of free sugar.

The data presented in Table I indicate a definite correlation between sedimentation rate and the amount of precipitate formed in plasma by the growth of Type III pneumococcus.

Discussion. According to Friedemann and Sutliff,[3] the precipitate formed in sera inoculated with pneumococcus is due to the fermentation of an abnormal polysaccharide only found in blood from certain disease conditions. The relation of the amount of precipitate and the sedimentation rate, as indicated in the present report, suggests that the abnormal carbohydrate may be responsible, at least in part, for the increased sedimentation rate of pathologic blood specimens. However, it must be recognized that increased sedimentation rate and large quantities of non-specific polysaccharide may occur simultaneously in the same blood specimen without having any bearing on each other. It is noted that similar correlations have been made by other workers between the fibrinogen or globulin level of the plasma and the sedimentation rate.

[4] Westergren, A., *Am. Rev. Tuberc.*, 1926, **14**, 94.

Recently, Seibert and her coworkers[5] have reported that a polysaccharide distinct from tubercle bacillus carbohydrate can be detected in the sera of individuals with tuberculosis. The extent of tuberculosis can be correlated with the amount of this polysaccharide in the serum. Interestingly enough, for many years clinicians have recognized the value of the sedimentation rate as an index of the progress of tuberculosis as well as other disease conditions. From the data given in the present report it is apparent that there is a definite relationship between sedimentation rate and an abnormal, non-specific polysaccharide in the plasma, and further work is planned to attempt to determine whether or not this polysaccharide is related to the carbohydrate investigated by Seibert and her coworkers.

Summary. A relationship existed between the sedimentation rate of blood from various disease conditions and the amount of precipitate formed in plasma by the growth of Type III pneumococcus. The possible importance of the presence of an abnormal, non-specific polysaccharide to this finding is discussed.

5 Seibert, F. B., Nelson, J. W., and Seibert, M. V., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 219.

14574

Effects of Pectin and Saline Solutions on Survival Time of Dogs in Hemorrhagic Hypotension.*†

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Recent observations of Middleton and Wiggers[1] indicated that intravenous infusions of pectin solutions were generally ineffective when given more than 30 minutes after reduction of mean arterial pressure to a sustained 50 mm Hg. level of arterial pressure. Given before the lapse of such an interval, but when significant hemodilution had already occurred, they caused a further immediate decrease in hemoconcentration due to the fluid injected. However, evidence that additional dilution or increase in blood volume occurs as a result of osmotic attraction of water was not discovered. On the contrary, a slight recovery of concentration in arterial blood generally supervened. Under such conditions, 8 out of 19 dogs reinfused at the end of a 30-minute period of hypotension failed to show a satisfactory dynamic response and died, but the remaining 11 showed a sustained elevation of arterial pressure and pressure pulses of good form from 4 to 6 hours after infusion of pectin solutions. But assuming that these animals would have recovered, only 58% would have been rescued by a comparatively prompt infusion of such solution.

The present investigation, a continuation of these studies, endeavored to determine the actual survival time when animals were transfused somewhat differently and under experimental conditions which might be expected to result in more favorable actions. For example, it is conceivable that most of the readily mobilizable body water has been drawn into the vascular system at the end of a 30-minute period of hypotension, with the result that no or little additional fluid can be reabsorbed when pectin solution with high colloid osmotic pressure is infused. For these reasons, it was felt that such infusions might act more favorably if more water were made available for reabsorption. To accomplish this

* This investigation was supported by a grant from the Commonwealth Fund.

† Condensed report of results presented as a thesis in partial fulfillment of requirements for the degree M.S. in the Graduate School of Western Reserve University.

1 Middleton, S., and Wiggers, C. J., *Am. J. Physiol.*, 1943, **140**, 326.

the animals received substantial subcutaneous infusions of physiological saline solutions during the experimental period. In addition, it seemed desirable to compare the results following infusion of pectin solutions with those which followed when no infusion was given or similar volumes of mammalian Ringer's solution were administered.

Procedures. As in previous experiments, dogs were anesthetized with morphine sulfate (20-40 mg) followed by sodium barbital, given intravenously (170-180 mg/kilo). The animals were bled rapidly until mean arterial pressure started to decline, then at a rate of 50 cc/min until it reached 60 mm Hg., and thereafter at a rate of 10-15 cc/min until a 50 mm Hg. level was reached. Here it was maintained—if necessary by additional small withdrawals of blood—for 30, 60, or 90 minutes. At the end of such periods of hypotension, one group of 10 animals received no treatment, a second group of 13 animals received infusions of mammalian Ringer's solution, and a third group of 29 dogs were infused with pectin solutions. Of the latter, 6 received a 1% pectin solution supplied by Dr. F. W. Hartman of Detroit and 23 received a 1.5% solution of pectin furnished by Frederick Stearns and Company of Detroit.

Just before use, these solutions were adjusted to pH 7.0 by addition of a 5% Na_2CO_3 solution (*ca.* 1 cc per 40 cc pectin solution). This change from phosphate or lactate buffers to a carbonate was made in accordance with suggestions from several sources that the former might conceivably have deleterious actions in states of hypotension.

Infusions of mammalian Ringer's or pectin solutions were given at rates of 20-40 cc/min. until blood pressures failed to increase further. Experience had shown that less volume of an infusate is required to achieve this result at more rapid rates of infusion and that dogs during post-hemorrhagic hypotension tolerate a more rapid rate of pectin infusion than have been suggested for normal dogs (3-5 cc/min[2]). Frequently, normal blood pressure was restored by infusing a volume of liquid less than that of the blood lost (*partial infusions*), but in some animals an equal volume was required (*full infusions*). When arterial mean pressure was not maintained above 80 mm Hg. after a partial infusion, a supplementary infusion was given, but the total volume of infusate never exceeded that of the blood which had been lost.

After such infusion—or after a 30-minute period of maintained hypotension in the case of untreated animals—mean arterial pressure was recorded for a minimal period of 4 hours. If at the end of such an observational period it tended to decline, the observation period was extended; if the pressure appeared to remain sustained the exposed femoral arteries were ligated, the wounds were packed with sulfanilamide and repaired and the animals were placed in a cage. If they lived, mean arterial pressures were recorded periodically during the second day. Under conditions of experimentation in which asepsis was not practiced, a survival for 36 hours with mean arterial pressures of 80 mm Hg. or more could fairly be considered to indicate recovery. However, many of these animals were not sacrificed for several days. Such animals ate and drank and used their hind limbs despite ligation of the femoral arteries.

Results. The results obtained on 50 dogs are summarized in Table I.

In view of the unfavorable responses to pectin solutions after a 60-minute interval of post-hemorrhagic hypotension and in view of previous unfavorable results reported by Middleton and Wiggers[1] there appeared to be no need of testing pectin solutions after 90-minute periods of hypotension. The results permit 3 deductions:

1. Anesthetized dogs submitted to a period of 50 mm Hg. post-hemorrhagic hypotension for only 30 minutes, as a rule, do not survive when untreated. In 90% of the dogs a state of circulatory failure develops which has all the dynamic characteristics of shock and leads to death by the following morning.

2. Infusion of pectin solutions materially increases the chance of survival but only when administered during an interval of 50 mm Hg. hypotension which does not exceed approxi-

[2] Hartman, F. W., Schelling, Victor, Brush, B., and Warren, K. W., *J. Am. Med. Assn.*, 1943, **121**, 1337.

TABLE I.

	No. of recoveries	No. of deaths	% recoveries
After 30 min. period of 50 mm hypotension			
No infusion	1	9	10
Ringer's sol.	5	2	71
Pectin "	8	5	61
After 60 min. period of 50 mm hypotension			
Ringer's sol.	4	2	67
Pectin "	3	8	27
After 90 min. period of 50 mm hypotension			
Ringer's sol.	0	3	0

mately 30 minutes. Supplementary subcutaneous injections of saline do not appear to extend the time of effectiveness. The dogs which survived showed no ill effects from a possible retention of a foreign colloid in the body, nor were gross evidences of organic disturbances seen at autopsy.

3. Pectin solutions seem to have no advantage over simple saline solutions; indeed, the latter act more favorably when administered after a 60-minute period of 50 mm Hg. hypotension.

These results, contrary to general belief, suggest that after severe hemorrhage the introduction of salt ions may be more important than the injection of foreign colloids. The reasons for this remain obscure. However, saline solutions lose their effectiveness after the deleterious effects of prolonged hemorrhage—in 3 cases 90 minutes of 50 mm Hg.—have become operative. This again stresses the fact that saline solutions must be used promptly after severe hemorrhage in order to exert any beneficial effects.

Summary. Results on 50 dogs submitted to variable periods of 50 mm Hg. post-hemorrhagic hypotension confirm the conclusions of Middleton and Wiggers[1] that infusions of pectin solutions do not materially increase the chance of recovery unless they are given during an interval which does not exceed 30 minutes of such hypotension. They extend these observations in showing that these beneficial effects are certainly no better than those achieved by administration of simple saline solutions. Finally, they once more stress the fact that the latter are not wholly useless provided they are administered before deleterious effects of prolonged hypotension become operative.

14575

The Antithyroidal Substances of the Blood and Vitamin A.

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An extensive literature has appeared which has confirmed the findings of Anselmino and Hoffmann[1] concerning the ether-soluble substances of the blood which have a pronounced antithyroidal effect and also are effective in animal experiments, as well as in the treatment of hyperthyroidism.

The present investigation was undertaken in order to determine the relationship of these ether-soluble substances of the blood to vitamin A. This question seemed important to us, since by animal experiments it had been previously demonstrated by one of us,[2] that vitamin A has a strong antithyroidal effect.

The comprehensive literature on the antithyroidal effect of vitamin A includes papers

[1] Anselmino, K. J., and Hoffmann, F., *Klin. Wchnschr.*, 1933, **12**, 99.

[2] Fellinger, K., and Hochstädt, O., *Wien. klin. Wchnschr.*, 1936, **49**, 1339.

TABLE I.
Relation of Extraction Time to Vitamin A Content and Antithyroidal Potency.

Time of extraction	1 hr.	2 hr	5 hr	15 hr
Vit. A content of ether-extr.	414 units/60 ml blood	No vit. A	No vit. A	No vit. A
Potency of antithyroidal substance of ether-extr.	No antithyroidal effect	No effect	Slight effect	Strong effect

by Abelin,[3] von Euler and Klussmann,[4] Schneider,[5] Falta,[6] Fleischmann and Kann,[7] Wendt[8] and others. Because this literature has undoubtedly shown the antithyroidal effect of vitamin A, its relationship to the antithyroidal substances of the blood was questioned since both must be removed from the blood simultaneously during ether-extraction. We therefore assayed the antithyroidal extract for its vitamin-A content and determined whether the antithyroidal effect may be caused or increased by the presence of vitamin A.

Methods. Blood from normal individuals, with no thyroidal disturbance was extracted in the Soxhlet with ether for varying lengths of time. The extract was distilled under reduced pressure to remove the solvent and the residue was dissolved in Wesson oil. This solution was assayed spectroscopically for its vitamin-A content.* The antithyroidal effect of the extract was determined by the method of Fellinger and Hochstädt,[9] a modification of the Reid Hunt technic.[10] In this modification thyroxin, which increases the resistance of white mice to acetonitril-poisoning quite markedly, is bound by the antithyroidal substances. Thus, the mice, no longer protected, succumb to the acetonitril.

Results. In our first experiments, blood was extracted with ether for 15 hours or longer. The extract always showed a strong antithyroidal effect on white mice, but vitamin A could not be demonstrated in any of the extracts. In successive series of experiments, it was found that after as little as 2 hours of extraction, there was no demonstrable vitamin A in the extracts. A moderate amount of vitamin A could be demonstrated only in experiments in which the extraction did not exceed one hour. In Experiment 6, for example, 60 ml of blood was extracted for 55 minutes and the extract contained approximately only 414 units of vitamin A. The normal average vitamin-A content of this quantity of blood should be about 4000 units. No vitamin A could be demonstrated in the residue of blood after extraction. Moreover, hardly any antithyroidal effect could be demonstrated after one hour of extraction.

Table I shows the relation between the extraction-time, the vitamin-A content and the antithyroidal content of the ether-extract.

Discussion. In review, we can say that the antithyroidal substances of the blood do not contain vitamin A. The antithyroidal substances were obtained by hours of extraction in the Soxhlet, a method in which the vitamin A is completely destroyed. Since we know that the destruction of vitamin A occurs in the presence of oxygen, we have to assume, that, in our procedure, the destruction of the vitamin A is accomplished rather rapidly in the receiving flask. Another possibility for the rapid destruction of vitamin A is the fact that this vitamin becomes more susceptible to oxidation when removed from the antioxidants which are present in the blood. We also found a strong antithyroidal effect of the ether-extract after 15 hours of extraction, a slight antithyroidal effect after 5 hours and no effect after less than 5 hours of extraction.

The authors thank Dr. Stephen Maddock, head of the Surgical Research Laboratory of the Boston City Hospital for his help and interest.

[3] Abelin, I., *Biochem. Z.*, 1930, **228**, 165

[4] Von Euler, H., and Klussmann, E., *Z. f. physiol. Chem.*, 1932, **213**, 21.

[5] Schneider, E., *Deutsche Z. f. Chir.*, 1934, **242**, 189.

[6] Falta, W., *Wien. klin. Wchnschr.*, 1935, **48**, 382.

[7] Fleischmann, W., and Kann, S., *Wien. klin. Wschnschr.*, 1936, **49**, 1488.

[8] Wendt, H., *Med. Klin.*, 1936, **32**, 27.

* The kind help of the Distillation Products, Inc., Rochester, N.Y., in the spectroscopical assay is gratefully acknowledged.

[9] Fellinger, K., and Hochstädt, O., *Klin. Wchnschr.*, 1935, **14**, 1250.

[10] Hunt, R., *Am. J. Physiol.*, 1922-23, **63**, 257.

14576

Excretion of a Methemoglobin-forming Substance in Urine.

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In a peculiar instance of methemoglobin formation first differentiated by Stokvis[1] as a clinical entity "enterogenous cyanosis methemoglobinemia" the urine was shown to contain a substance which was able to convert hemoglobin into methemoglobin *in vitro*. During the course of an investigation of the oxidation-reduction potentials of concentrated dialysates of *Streptococcus viridans* cultures[2] we found that these dialysates were able to form methemoglobin if added to blood, while the original culture medium had no such effect. It therefore occurred to us that the urine of patients suffering from sub-acute bacterial endocarditis might also have this property. On adding such a urine to a dilute solution of blood, the blood turned chocolate brown and the typical spectroscopic bands of methemoglobin became visible.

On the supposition that the urinary constituent causing the formation of methemoglobin must be a relatively high oxidant we tried its action on an acidified solution of potassium iodide and found it capable of liberating free iodine; the urine turned yellowish-brown, and, on the addition of starch, a deep purplish blue. This provided a quick method for the qualitative detection of the methemoglobin-forming substance.

These urines had the following properties in common: 1. On addition to diluted blood there was rapid formation of methemoglobin and the typical spectral bands appeared. 2. On addition of di-methyl *p*-phenylenediamine in acetic acid solution there was instantaneous formation of the bright scarlet red color of Würster's Red. 3. On addition of benzidine in acetic acid solution a bright orange color developed. 4. On addition to a solution containing equal volumes of 10% KI and 2N H_2SO_4 the solution turned yellow, and with starch, deep blue. 5. In an atmosphere of nitrogen, concentrates of the urine restored the color to leuco indigo carmine, leuco methylene blue and leuco 2-6-dichlorphenolindophenol. 6. Owing to continued reoxidation of the dichlorphenolindophenol the ascorbic acid content of positive urines could not be determined accurately by the standard titration method. The results obtained were very low. 7. The active substance was steam-distillable and freely diffusible.

All these reactions are reproducible with water-soluble quinones of the same concentration, including 2-methyl-1,4 naphthoquinone, the vitamin K analogue.

Quantitative Determination of the Methemoglobin-forming Substance as Quinone. To 20 cc of urine, 10 cc of 10% KI and 10 cc of 2N H_2SO_4 are added. The solution turns yellowish brown. This is titrated with n/100 sodium thiosulfate and just before the endpoint a few drops of freshly prepared soluble starch are added as an indicator. The end point is extremely sharp and is reached when the blue color disappears. The cc thiosulfate $\times$ 2.7 = mg substance as quinone in 100 cc.

Urine showing a quinone titer of 8.5 mg % was added to blood diluted 1:4, to determine the amount of methemoglobin formation by the decrease in oxygen capacity measured by the manometric method of Van Slyke. (Table I.) When the specimen was dialyzed through a collodion membrane and the dialysate concentrated *in vacuo* the quinone equivalent was raised to 29.8 mg % and the amount of methemoglobin formed was markedly increased.

Since these urines oxidize leuco dichlorphenolindophenol the usual titration method of determining ascorbic acid by reducing this

[1] Stokvis, B. J., *Nederl. Tijdschr. Geneesk.*, 1902, **2**, 678.

[2] Fishberg, E., and Baum, H., *J. Biol. Chem.*, 1938, **123**, xxxv.

TABLES I AND II.
Formation of Methemoglobin by Urine Containing 8.5 mg % Quinone Equivalent.

Blood, cc	Urine, cc	Water, cc	Oxygen cap. vol. %	Meth. Increase vol. %	Meth. % increase	Meth. spect.
2	0.0	2.0	19.34	0	0	0
2	0.25	1.75	18.49	0.85	4.4	+—
2	0.50	1.50	17.91	1.42	7.4	++
2	0.75	1.25	17.11	2.23	11.5	++
2	1.0	1.0	16.01	3.33	17.2	+++
2	1.3	0.7	15.02	4.31	22.3	+++
2	1.6	0.4	13.97	5.37	27.8	++++
2	2.0	0.0	12.99	6.34	32.8	>++++
Formation of Methemoglobin by Dialyzed Urine Containing 29.8 mg % Quinone Equivalent.						
2	0.0	2.0	19.93	0	0	0
2	0.4	1.6	15.99	3.93	19.7	+++
2	0.7	1.3	13.96	5.97	29.9	++++
2	1.0	1.0	10.66	9.27	46.5	>++++
2	1.4	0.6	8.84	11.09	56.7	>++++
2	1.7	0.3	7.27	12.66	63.5	>++++
2	2.0	0.0	5.00	14.93	74.9	>++++

TABLE III.
Influence of Methemoglobin-forming Material on the Titration of Ascorbic Acid.

Ascorbic acid present, mg	Dichlorphenol indophenol, cc	Urine added, cc	Quinone by titration, mg	Ascorbic acid found, mg/100 cc	% found of ascorbic acid present
.0176	.73	0	0	17.6	100
.0176	.60	0.2	.018	14.47	82.2
.0176	.535	0.3	.028	12.90	73.3
.0176	**.42**	**0.5**	**.045**	**10.13**	**57.5**
.0176	.30	0.7	.064	7.24	41.2
.0176	.125	1.0	.091	3.01	17.1
.0176	.071	1.1	.100	1.71	9.7

dye to its leuco form obviously cannot be employed. Methemoglobin-forming urines titrated for ascorbic acid showed a daily output of 1.3, 1.66, 1.1, and 2.1 mg in contrast to non-methemoglobin-forming urines which showed the normal values of 10-25 mg. For a direct demonstration varying quantities of positive methemoglobin-forming urine were added to 0.1 cc of a solution of ascorbic acid containing 17.6 mg % and the mixture was then titrated with dichlorphenolindophenol. As seen from Table III, the concentration of ascorbic acid so measured is an inverse linear function of the amount of positive urine and does not in any way represent the amount of ascorbic acid actually present.

Over 9,000 specimens sent to the routine laboratory from the wards were tested for the presence of the methemoglobin-forming substance. Those which were positive over periods of several days and showed values between 3 and 10 mg % were from cases of sub-acute bacterial endocarditis, rheumatic fever, and acute arthritis. There were negative intervals between the positive periods. In a special group of which we had seven cases the excretion of the methemoglobin-forming substance was continuous and showed extremely high values up to 40 mg %. These included a 69-year-old man admitted for continued epistaxis who had subsisted on a diet completely free of fruits and vegetables for a period of over 40 years, an infant suffering from acute scurvy, and 2 cases of pneumococcus peritonitis in children following nephrosis. The urines of both these cases were continuously negative over a stay of several months in the hospital but turned

positive for the methemoglobin-forming substance about one week before the blood culture became positive for pneumococci. There were also two cases of hemorrhagic colitis.

Summary. A substance capable of forming methemoglobin from blood *in vitro*, and which can be quantitatively estimated as quinone, has been found to be excreted in certain pathological states. This urinary constituent interferes with the titration of ascorbic acid.

14577 P

Influence of Pituitary Adrenotrophic Hormone on Lymphoid Tissue Structure in Relation to Serum Proteins.*

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Lymphoid tissue mass[1,2] and blood lymphocytes[3] are under pituitary adrenotrophic hormone influence. Loss in lymphoid tissue weight and the lymphopenia following hormone injection suggested an approach to the problem of the fate of lymphocytes.

This paper presents a preliminary description of lymphoid tissue histology after injections of adrenotrophic hormone into normal mice and rabbits. Histological alterations observed suggested study of effects of adrenotrophic hormone on serum proteins and antibody formation.

Histology. Single, subcutaneous injections of 1 mg adrenotrophic hormone[4] in 0.5 ml of water into mice or 10 mg in 2 ml solution into rabbits produce alterations in lymphoid tissue histology. Within 1 to 3 hours, degenerative changes are evident in lymphocytes in germinal centers of lymph nodes, in Malpighian follicles of the spleen, in the thymic cortex, and in Peyer's patches. Three to 6 hours after injection, depletion of lymphocytes in lymphoid tissue occurs, accompanied by marked edema. Degenerative changes are characterized by pycnosis and nuclear fragmentation in small or medium-sized lymphocytes. Phagocytosis of nuclear debris and red cells is accompanied by polymorphonuclear invasion of lymphoid tissues. Restoration of normal lymphoid structure begins after 9 hours and is still continuing 24 hours after hormone injection. These changes do not occur in adrenalectomized mice injected with adrenotrophic hormone.

The loss of lymphocytes from lymphoid tissue and from the circulation, and their absence from large capillary beds, suggested dissolution of these cells. In this event, significant quantities of protein nitrogen would be released for metabolic use. A portion of the protoplasmic nitrogen of the dissolved lymphocytes was sought in the serum proteins.

Serum Protein Observations. Total serum proteins and the specific gravity of whole blood were determined on tail vein blood by Kagan's methods.[5,6] No significant alterations in whole blood specific gravity occurred. The serum protein data are presented in

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† Fellow of the International Cancer Research Foundation.

1 Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 132.

2 Simpson, M. E., Li, C. H., Reinhardt, W. O., and Evans, H. M., *Ibid.*, 1943, **54**, 135.

3 Dougherty, T. F., and White, A., *Science*, 1943, **98**, 367.

4 Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

5 Kagan, B. M., *J. Clin. Invest.*, 1938, **17**, 373.

6 Kagan, B. M., *J. Lab. and Clin. Med.*, 1941, **26**, 1681.

TABLE I.
Effect of Pituitary Adrenotrophic Hormone and of Adrenalectomy on Total Serum Proteins.

Animal	No. of animals	Treatment	Total serum proteins, g/%	P values†
Normal rat	22	None	6.00 ± 0.01*	
" "	8	3 hr after 3 mg A‡	6.22 ± 0.07	<0.01
" "	8	6 " " " " "	6.30 ± 0.08	<0.01
" "	4	24 " " " " "	5.81 ± 0.01	0.04
" mouse	19	None	6.02 ± 0.02	
" "	17	1 mg A daily, 15 days	6.52 ± 0.07	<0.01
Adrenalectomized mouse	16	0.025 mg D§ daily, 8 days	5.59 ± 0.08	<0.01

* Means and standard errors.
† P values, compared to controls.
‡ A = adrenotrophic hormone.
§ D = desoxycorticosterone acetate in oil (Schering).

Table I. After a single injection of adrenotrophic hormone, total serum proteins increase at the time lymphoid tissues undergo involution and lymphopenia occurs. Twenty-four hours after injection, the lymphoid tissue and blood lymphocyte count are almost normal,[3] coinciding with approximately control serum protein values. Chronic hormone injections result in decreased lymphoid tissue weight,[1,2] a trend toward persistent lymphopenia,[3] and consistently elevated serum protein levels. Adrenalectomized mice, receiving desoxycorticosterone daily, show enlarged lymphoid tissues,[7] a lymphocytosis,[7] and subnormal serum protein concentration. In normal or adrenalectomized mice, the dose of desoxycorticosterone used produces no lymphopenia[7] or lymphoid tissue involution.[7,8] However, this dose is sufficient to prevent hemoconcentration in the adrenalectomized mouse. In the absence of desoxycorticosterone administration, hemoconcentration obscures the serum protein fall.

Comment. Injection of adrenotrophic hormone, or cortical extract, produces an absolute lymphopenia. A probable basis for the peripheral lymphopenia is the dissolution of lymphocytes in tissues following injection of either hormone. Since increased serum proteins occur simultaneously with washing out of lymphocytes as a result of adrenotrophic hormone action,‡ lymphoid tissue is a possible source of serum proteins.

The alterations observed in serum proteins (Table I) do not appear to be of great magnitude, although highly significant. However, when these changes are considered in the light of the circulation rate, and the period over which they persist, it is apparent that an increased quantity of protein is being added to the blood. A portion of this protein may undergo glyconeogenesis in the liver. Since the following communication[9] establishes that adrenal cortical extract injection increases antibody titer, it is apparent that the globulins are one fraction of the serum proteins which were augmented in the present study.

Summary. Decrease of lymphocytes in lymphoid tissue and the concomitant lymphopenia following injection of pituitary adrenotrophic hormone, or adrenal cortical extract, are in their time relationships correlated with an increase in serum proteins. These same relationships persist in animals given daily injections of adrenotrophic hormone. Adrenalectomized mice, in which hemoconcentration is prevented, have a lower than normal concentration of serum proteins for at least 8 days following operation.

7 Unpublished results.

8 Wells, B. B., and Kendall, E. C., *Proc. Staff Meetings Mayo Clinic,* 1940, **15**, 133.

‡ The changes in lymphoid tissue structure, in blood lymphocytes and in serum proteins are essentially the same if adrenal cortical extract is used in place of adrenotrophic hormone in normal animals.

9 Dougherty, T. F., White, A., and Chase, J. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 28.

14578 P

Relationship of the Effects of Adrenal Cortical Secretion on Lymphoid Tissue and on Antibody Titer.*

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Recent support for the hypothesis that antibodies are produced in lymphoid tissue has been obtained by Ehrich and Harris.[1] Since the injection of pituitary adrenotrophic hormone, or adrenal cortical extract, produces lymphoid tissue dissolution concomitant with serum protein increases,[2] a study was made of the effect of adrenal cortical extract on antibody titers.

Experimental and Results. Twelve rabbits (3 months old) received daily intravenous injections of sheep erythrocytes. Four of these animals also received daily subcutaneous injections of 2 ml of aqueous adrenal cortical extract (Wilson). Agglutinin titers were done periodically throughout the experiment. This experiment was terminated when injection of antigen alone did not result in further titer increases. These data are presented in Fig. 1.

All rabbits receiving adrenal cortical extract with antigen show higher final agglutinin titers than rabbits injected with antigen alone. Adrenal cortical extract produced augmentation of titer late in the experimental period.

Nine rabbits with maximum titers were used to study the effect of single injections of hormone. The experimental procedures, together with the data, are presented in Table I. A single injection of antigen alone did not increase the titer within 25 hrs. Animals injected with 5 ml aqueous adrenal cortical extract, with or without antigen, showed definite increases in titer in 6 hours. Rabbits receiving 10 ml aqueous extract, or 5 ml of adrenal cortical steroids in oil,‡ showed similar augmentation. The titers were lower at 11 hours, and at 25 hours were almost at pre-injection level.

Two rabbits (A 40 and A 41) were injected daily with 1 ml of cortical steroids in oil; no further antigen was administered. After 2 weeks, titers done approximately 15 hours following the last injection were higher than those before hormone treatment.

Comment. A definite time relationship exists among changes in lymphoid tissue structure,[2] in blood lymphocyte levels,[3] in serum protein concentration,[2] and in antibody titers following a single injection of adrenotrophic hormone or of adrenal cortical extract. Chronic adrenotrophic hormone treatment diminishes lymphoid tissue,[4] produces a consistent lymphopenia[3] and increases serum protein levels.[2] Data presented here show that one of the augmented serum protein fractions is that containing antibodies. These interrelationships are normally under pituitary adrenotrophic hormone influence and may be altered by a variety of stimuli affecting pituitary-adrenal cortical activity.

Summary. Agglutinin titers to sheep erythrocytes are enhanced as a result of adrenal cortical extract injection. Titers were increased within 6 hours after a single injection of adrenal cortical extract into hyperimmunized rabbits. Increased titers produced by a single injection of adrenal cortical

* This investigation was aided by grants from the Josiah Macy, Jr., Foundation and the Fluid Research Fund of Yale University School of Medicine.

† International Cancer Research Foundation Fellow.

1 Ehrich, W. E., and Harris, T. N., *J. Exp. Med.*, 1942, **76**, 335.

2 White, A., and Dougherty, T. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 26.

‡ Grateful acknowledgment is made to Dr. E. Gifford Upjohn of the Upjohn Company for supplying the preparation of adrenal cortical steroids in oil.

3 Dougherty, T. F., and White, A., *Science*, 1943, **98**, 367.

4 Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 132.

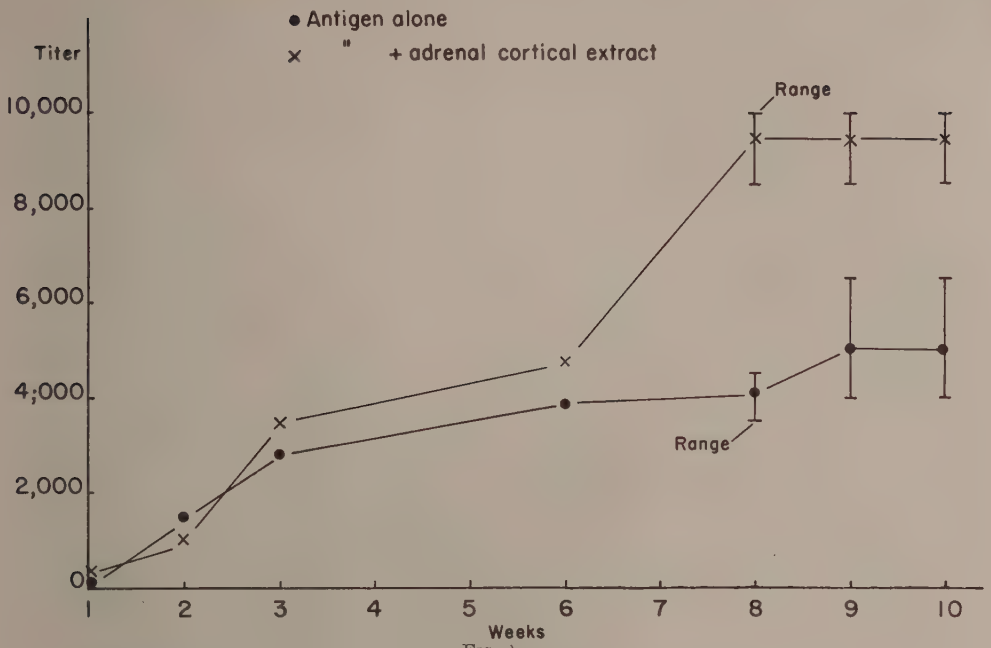

FIG. 1.

Average agglutinin titers of 8 rabbits receiving, daily, intravenous injections of sheep erythrocytes alone, and of 4 animals injected with antigen and adrenal cortical extract.

TABLE I.

Effect of Single Injection of Adrenal Cortical Extract on Sheep Erythrocyte Titers in Rabbits.

Rabbit No.	Treatment	Agglutinin titers, time after injection					
		0 hr	6 hrs	11 hrs	25 hrs	1 wk	2 wks
A 39	10 ml 10% erythrocyte suspension	1-6500	1-6500	1-6500	1-6500	1-8000	1-7500
A 38	5 " aqueous cortical extr.	1-5000	1-8000	1-6000	1-6000	1-5000	1-4500
A 34	5 " " " "	1-4500	1-9000	1-7000	1-5500	1-5000	1-5000
A 33	5 " " " " + 10 ml 10% erythrocyte susp.	1-3500	1-6000	1-6000	1-5000	1-4000	1-3500
A 36	5 ml aqueous cortical extr. + 10 ml 10% erythrocyte susp.	1-5000	1-8500	1-6500	1-5000	1-5000	1-4500
A 42	10 ml aqueous cortical extr.	1-8000	1-12,000	1-10,000	1-8500	1-7500	1-7500
A 31	10 " " " "	1-10,000	1-15,000	1-12,000	1-10,000	1-10,000	1-10,000
A 40	5 ml cortical steroids in oil; then 1 ml daily for 16 days	1-8500	1-15,000	1-15,000	1-11,500	1-10,000	1-11,500
A 41	5 ml cortical steroids in oil; then 1 ml daily for 16 days	1-9500	1-17,000	1-17,000	1-15,000	1-11,500	1-13,000

hormone return in 24 hours almost to levels existing before hormone administration. Continued hormone injection maintained elevated titers for 2 weeks.

14579

White Blood Cell Preservation with Yeast Extract in Stored Blood.

EILEEN W. E. MACFARLANE. (Introduced by Elton S. Cook.)

From the Department of Experimental Medicine, Institutum Divi Thomae, Cincinnati, Ohio.

The importance of vitamins of the B-complex in relation to blood formation and phagocytosis is now well recognized. Drew, Scudder and co-workers[1,2,3] investigated the fate of cellular elements in stored blood and found that the most rapid deterioration occurred in the polymorphonuclear neutrophilic leucocytes which diminished 50% in 48 hours in heparinized and citrated preserved blood. It was therefore decided to test the effect of yeast extract on white blood cells in citrated blood.

Method. The yeast extract was dissolved in 5% sodium citrate; the final concentrations in the stored blood were 0.5% citrate and 2.2% yeast extract. The yeast extract was a purified alcoholic extract of Fleischmann's baker's yeast corresponding to Sample A as described by Cook, Kreke, and Nutini,[4] and was obtained from them.

Twenty cubic centimeters of freely flowing venous blood were divided in half; each 10 cc portion was mixed with 1 cc of citrate, one with yeast extract and one without. Each mixture was distributed into 10 sterile test-tubes (10 x 75 mm), 1 cc into each. The tubes were stored at approximately 4°C. One tube was taken daily from each series and the cells were well mixed by rotating and inverting by hand. Total white cell counts and differential counts on smears stained with Wright's stain were then made. Experiments were run with blood from 3 donors.

Results. The total white cell count in blood (donor J. F.) with 2.2% yeast extract fell only a little over 6% in the first 3 days. This loss was almost all in the neutrophilic leucocytes (Fig. 1). In control samples there was a fall of 28% and 23% respectively in total white count in the first 24 hours. The diminution of neutrophiles was of the same order and not as rapid as in Drew's and Scudder's experiments. This may have been due to the smaller samples and narrower tubes. After 4 days with yeast extract the neutrophiles had decreased by 50%, but the lymphocytes and monocytes showed total losses of only 15 to 25%. After one week the total white count of controls was about the same as in Drew and Scudder's report. In the treated blood the total white count remained slightly but consistently higher from the end of the first week to the end of 30 days.

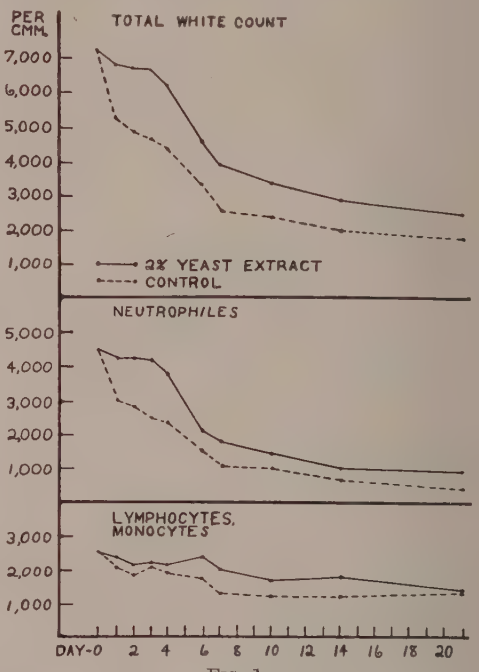

FIG. 1.
Cellular changes in citrated blood with and without 2.2% yeast extract. Donor J.F.

[1] Drew, C. R., Edsall, K., and Scudder, J., *J. Lab. and Clin. Med.*, 1939, **25**, 240.

[2] Drew, C. R., and Scudder, J., *J. Lab. and Clin. Med.*, 1941, **26**, 1473.

[3] Scudder, J., Drew, C. R., Tuthill, E., and Smith, M. E., *Bull. N. Y. Acad. Med.*, 1941, **17**, 373.

[4] Cook, E. S., Kreke, C. W., and Nutini, L. G., *Studies Inst. Divi Thomae*, 1938, **2**, 23.

Trager[5] found that concentrations of 0.7% and 0.14% of yeast autolysate improved the survival of malaria parasites *in vitro*, with 0.7% as optimum. Another experiment was therefore run in the same manner with blood from donor D.D. This blood was divided into 3 portions, including a control set, with final concentrations of 1.1% and 0.22% of yeast extract in 0.5% sodium citrate. This blood showed a somewhat high original total white count of 9,500 which had fallen to 3,000 on the sixth day in the control. A marked difference was shown between the white cell population in the 3 sets during the first 5 days. In the control the total white count fell by 2,300, which was only 23%, and was then held at this level for 2 more days before it began to fall rapidly. The blood with 1.1% yeast extract maintained the white cell population with scarcely any loss for 48 hours. They then decreased gradually for 2 more days, after which their total fell quickly. The daily total white cell count with 0.22% yeast extract was higher than in the control but less than with 1.1%; it fell gradually by 17% up to the third day, and was then arrested for 24 hours before falling rapidly as in the other sets. By the end of a week there was no appreciable difference between this blood and the control; but the blood with 1.1% yeast still showed a total white cell count 25% higher than the control.

In all experiments the decrease in total white cell count was found to be due mainly to loss in polymorphonuclear neutrophilic leucocytes and the 2 curves were always similar to those shown in Fig. 1. The lymphocytes, in contrast, decreased very gradually and by the third or fourth day they seemed to show a slight increment over the first and second day; this is also evident in the curves of Drew and Scudder. After one week they remained fairly constant at about 75% of their original number in blood with 2.2% and 1.1% yeast extract and at about 50% of their original strength in the controls.

Other Cellular Elements. Observations on monocytes, eosinophiles, and basophiles confirmed the findings of Drew and Scudder.[1,2] The platelets and erythrocytes were not investigated. In all experiments, after 3 or 4 weeks there was noticeably less hemoglobin in the plasma with yeast extract than in controls.

Summary. The addition of 2.2% and 1.1% of yeast extract to citrated blood kept the total white count close to normal for 3 or 4 days. The improvement was due chiefly to a delay in the disintegration of the neutrophilic leucocytes, but the number of lymphocytes also remained consistently somewhat higher than in the controls for a month.

[5] Trager, W., *J. Exp. Med.*, 1943, **77**, 411.

14580 P

Observations on Morphological Changes in Leucocytes of Stored Blood.

EILEEN W. E. MACFARLANE. (Introduced by Elton S. Cook.)

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Amoeboid Movement of Leucocytes. There is some doubt whether the leucocytes in stored blood are still alive and functional. After 14 days storage with 2% yeast extract the white cell layer was removed from a test tube of blood (donor E.M.) and mixed with a drop of brilliant cresyl blue, a supra-vital stain that dyes the nucleus. Examined microscopically at about 25°C the damaged cells so evident in smears were not seen; many of the leucocytes showed active amoeboid movement, others appeared dark, granular, and moribund. In blood of this age the polymorphonuclear leucocytes and the medium-sized lymphocytes have about the same size, but can be distinguished by the way they move. The former in old blood have band-like nuclei that change from U-shape to Y and J-shape; a portion may break off and later reunite with the rest of the nucleus. After 2 weeks the few neutrophiles left still move with the cytoplasm pushing ahead as described by Kubie and Schultz[1] in the cat. The lymphocytes put out narrow pseudopodia of nuclear material as described by Lewis[2] and by Bloom.[3]

Neutrophilic Leucocytes. Sabin and coworkers[4] found that there is a rapid periodic death of neutrophiles "in showers" in the circulating blood, but that this is not true of lymphocytes; about one-fifth of the neutrophilic leucocytes died every 24 hours. In the yeast extract experiments the neutrophiles decreased in the first day by about 25% of their original number in the controls and the total number of lymphocytes was maintained with a loss of only 23% to 28% for over 2 weeks. The polymorphonuclear leucocytes became rounded cells with a band or kidney-shaped nucleus and clear pale cytoplasm as observed by Clark, Clark, and Rex[5] in lizards and rabbits.

Lymphocytes. Normal size differences among lymphocytes remained throughout a month in stored blood. After one week there were more lymphocytes than neutrophilic leucocytes in all samples (3 different donors). The small lymphocytes with little cytoplasm are highly resistant; they are highly basophilic and resemble mature or senile lymphocytes of circulating blood[4] and the plasma cells of pathology (Bloom).[3] Yeast extract kept the lymphocyte count above that in the controls, with a slight increase in the total count sometimes between the fourth and the sixth day. These increases in leucocyte count may be due to an increment among the lymphocytes by an accretion from changed neutrophiles as Lewis[2] reported. Some of the smallest very dark staining lymphocytes in old stored blood may be naked nuclei such as were seen by Kubie and Schultz.[1]

Eosinophiles, as found by other workers, are the most resistant of all the granulocytes. As in the neutrophiles, the nucleus becomes rounded or kidney-shaped. The eosinophilic vacuoles enlarge and are very brilliant in blood 2 or 3 weeks old.

1 Kubie, L. S., and Schultz, G. M., *Johns Hopkins Hosp. Bull.*, 1925, **37**, 91.

2 Lewis, W. H., *Johns Hopkins Hosp. Bull.*, 1931, **49**, 29.

3 Bloom, W., in Downey, H., *Handbook of Hematology*, 1938, **1**, 375.

4 Sabin, F. R., Cunningham, C. A., Doan, C. A., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, **37**, 14.

5 Clark, E. R., Clark, E. L., and Rex, R. O., *Am. J. Anat.*, 1936, **59**, 123.

14581

A New *Salmonella* Type with Hitherto Undescribed Somatic Antigens.*

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Salmonella inverness, the type to be described, was isolated from the stool of a normal food handler by Mrs. Mildred Galton and Mr. M. S. Quan of the Florida State Department of Health. After classification as a member of the *Salmonella* group it was forwarded to the writers for identification.

The organism exhibited the usual morphological and cultural characteristics of the *Salmonella* group. It produced hydrogen sulfide but failed to liquefy gelatin or produce indol. Glucose, rhamnose, arabinose, xylose, maltose, trehalose, dulcitol, sorbitol, and inositol were fermented with the production of acid and gas. Lactose, sucrose, and salicin were not attacked. Dextro-tartrate, levo-tartrate, mucate, and citrate were decomposed but meso-tartrate was not utilized.

On serological examination it was found that *S. inverness* was not agglutinated by serums representative of the various somatic antigens of the Kauffmann-White classification. Likewise, a serum derived from a heated culture of *S. inverness* failed to react in significant degree with any of the known somatic antigens. It was obvious that *S. inverness* had a distinct somatic complex and to it the symbol XXXVIII was assigned.

Examination of the flagellar antigens of the new type revealed that it was diphasic. Phase 1 was flocculated to the titer of serum derived from phase 1 of *S. thompson* (k) and in absorption tests *S. inverness* removed all flagellar agglutinins from *S. thompson* serum. Phase 1 of *S. inverness* may be designated as k.

Phase 2 of *S. inverness* was agglutinated by serums for all the nonspecific phases of Kauffmann-White classification. When tested with single factor serums for antigens 2, 3, 5, 6, and 7, it was agglutinated only by 6 serum. In absorption tests phase 2 of *S. inverness* removed all agglutinins for phase 2 of *S. poona* (1,6 . . .) and phase 2 of *S. amherstiana* (1,6 . . .) from serum derived from phase 2 of *S. anatum* (1,6 . . .). A slight residue of agglutinins amounting to less than 2% of the original titer was left for the homologous strain. Phase 2 of *S. inverness* may be designated as 1,6 . . .

Summary. *S. inverness,* a new *Salmonella* type isolated from a normal food handler, was described. It possessed a hitherto undescribed somatic antigen and was assigned the antigenic formula XXXVIII:k-1,6 . . .

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

14582

Bacteriostatic Activity of Sulfonamides Against *B. morganii* and Paracolon Bacilli.

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During recent years suggestive evidence has been obtained indicating that *B. morganii* type I and paracolon bacilli may play a role in enterocolitis and gastroenteritis (Stuart, Wheeler, Rustigian, and Zimmerman,[1] Neter and Bender,[2] and others). It seemed of interest, therefore, to determine whether or not these organisms are susceptible to the action of sulfonamides *in vitro* as a preliminary study to an investigation on the chemotherapeutic effectiveness of these drugs *in vivo*. Sulfasuccidine seems to be the drug of choice at the present time in the treatment of localized enteric infections amenable to sulfonamide therapy. Since the *in vitro* activity of sulfasuccidine does not parallel its *in vivo* effectiveness, the following experiments were carried out with sulfanilamide and sulfathiazole.

Material and Methods. Several freshly isolated strains of *B. morganii* type I and paracolon bacilli were used in this study. The strains were recovered from the feces of infants and children with diarrheal disease. The biochemical reactions of the strains of *B. morganii* were characteristic of this species. The paracolon bacilli produced acid and gas from glucose, but failed to ferment lactose even when the incubation period was extended over 3 weeks.

The strains were grown for 24 hours at 37°C in infusion broth and tryptone, respectively. The bacteriostatic activity of sulfonamides was tested in 1% glucose-phenol red broth (Difco). This broth contained beef extract, protose peptone No. 3, sodium chloride, and phenol red. Sulfanilamide was added to give concentrations of 10 mg %, 100 mg %, and 1,000 mg %, and sulfathiazole to give a concentration of 100 mg %. Broth without sulfonamides was used as a control. These culture media were sterilized by autoclaving at 15 lbs pressure for 12 minutes. To 5 ml of these broths was added 0.2 ml of a 1:10,000 diluted broth culture of the respective microörganisms. The tubes were then incubated at 37°C and the resulting growth was noted at various intervals.

Results. Table I presents the results of the bacteriostatic action of sulfanilamide and sulfathiazole toward 3 strains of *B. morganii* and 3 strains of paracolon bacillus. It is evident from this table that these 6 strains were susceptible to the bacteriostatic activity of both sulfanilamide and sulfathiazole. Sulfathiazole in concentrations of 100 mg % was slightly more effective than sulfanilamide in like concentration. On the other hand, sulfanilamide in a concentration of 1,000 mg % exerted greater bacteriostatic activity than sulfathiazole in a concentration of 100 mg %. As a matter of fact, it permanently (7 days) prevented visible growth of all strains tested. In no instance was sulfanilamide in a concentration of 10 mg % effective, even after a period of only 6 or 8 hours. Eight additional strains of *B. morganii* type 1 and paracolon bacillus were tested. Essentially identical results were obtained.

Within the genus *Salmonella* there exist differences in susceptibility to sulfonamides among the different species (Bornstein and Strauss[3]). According to Bornstein,[4] sulfaguanidine showed a strong bacteriostatic action on *S. choleræ suis; S. paratyphi A* was fairly well inhibited. All other types were either entirely resistant or inhibited only by

[1] Stuart, C. A., Wheeler, K. M., Rustigian, R., and Zimmerman, A., *J. Bact.*, 1943, **45**, 101.

[2] Neter, E., and Bender, N. C., *J. Pediat.*, 1941, **19**, 53.

[3] Bornstein, S., and Strauss, L., Proc. Soc. Exp. Biol. and Med., 1941, **47**, 112.

[4] Bornstein, S., *J. Immunol.*, 1943, **46**, 429

TABLE I.
Bacteriostatic Activity of Sulfanilamide and Sulfathiazole Against *B. morganii* and Paracolon Bacillus.

Strain	Incubation at 37°C	Sulfanilamide 10 mg%	Sulfanilamide 100 mg%	Sulfanilamide 1,000 mg%	Sulfathiazole 100 mg%	Control
B. morganii						
No. 3411	6 hr	++	—	—	—	++
	24 "	++++	++++	—	++++	++++
	7 days	++++	++++	—	++++	
No. 3156	8 hr	++++	++++	—	—	++++
	24 "	++++	++++	—	—	++++
	7 days	++++	++++	—	++++	
No. 2688	8 hr	++++	++++	—	++	++++
	24 "	++++	++++	—	++++	++++
	7 days	++++	++++	—	++++	++++
Paracolon bacillus						
No. 3481	8 hr	++	—	—	—	++
	24 "	++++	++++	—	+	++++
	7 days	++++	++++	—	++++	
No. 2869	8 hr	++++	—	—	—	++++
	24 "	++++	++++	—	—	++++
	7 days	++++	++++	—	++++	
No. 2959	8 hr	++	—	—	—	++
	24 "	++++	++++	—	++	++++
	7 days	++++	++++	—	++++	++++

—: No visible growth.
+ to ++++: Various degrees of visible growth.

strong concentrations, much higher than those inhibiting the growth of *B. coli*. The possibility was considered that similar differences may exist between members of the genus *Escherichia* and the paracolon bacilli. To investigate this problem, Endo agar and SS (Salmonella-Shigella) agar with and without sulfanilamide were inoculated with feces from patients with diarrheal disease. Preliminary studies revealed that sulfanilamide inhibited both *B. coli* and paracolon bacilli. Thus, addition of this drug to selective and differential culture media does not produce a higher percentage of isolations of paracolon bacilli from fecal specimens.

Summary. (1) Sulfanilamide and sulfathiazole exert bacteriostatic activity toward *B. morganii* type I and paracolon bacillus. (2) Sulfathiazole in a concentration of 100 mg % is somewhat more effective than sulfanilamide in like concentration, but is less growth-inhibitory than sulfanilamide in a concentration of 1,000 mg %.

14583

Treatment of Dicoumarol-Induced Hypoprothrombinemic Hemorrhage with Vitamin K_1 Oxide.*

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Retardation of the rate of coagulation of human blood may be accomplished by the use of heparin or of dicoumarol (3-3′-methylenebis [4-hydroxycoumarin]). The action of heparin may be neutralized by protamine;[1] that of dicoumarol by vitamin K_1 oxide.[2] Under the influence of dicoumarol, spontaneous hemorrhages have been reported in cattle,[3] dogs,[4] and man.[5] In man the possibility of spontaneous hemorrhage following the administration of dicoumarol must not be overlooked, especially since in any given individual the tendency to bleed, and the time of appearance and intensity of the induced hypoprothrombinemia cannot be predicted. In our experience, a single large oral dose of dicoumarol (0.8 to 1.0 g) will produce an appreciable drop in the prothrombin concentration within 24 hours. The period of induction of satisfactory therapeutic hypoprothrombinemia may take 5 or more days, and the period of spontaneous recovery following discontinuance of the drug from 6 to 10 or more days.

Oral doses of dicoumarin varying between 150 and 300 mg fail to produce satisfactory changes in the plasma prothrombin concentration and coagulation time of the whole blood as tested by our methods.[6] Davidson and MacDonald[7] showed that the coagulation time of the blood, when tested in lusteroid tubes, was significantly prolonged before any alteration was demonstrable by the usual glass tube methods. The whole blood coagulation time, when tested in glass tubes (Lee and White method), does not appear to be significantly altered until the prothrombin concentration is depressed to 5% of normal. Therefore, in order to obtain a suitable prolongation of the whole blood coagulation time in the shortest possible period, we have used larger doses of dicoumarol (0.6 to 1.0 g daily) than those recommended in the literature.[8]

The hypoprothrombinemia induced by vitamin K deficiency is usually corrected by the administration of vitamin K compounds[9] that induced by dicoumarol appears to be refractory to the usual dosage of vitamin K and is only partially rectified by the transfusion of whole blood.[10,8,7] Davidson and MacDonald[2] reported that in human subjects, a very large dose of vitamin K_1 oxide will correct the hypoprothrombinemia produced by a single large dose of dicoumarol.

This report deals with the control of hemorrhage and the correction (by the administration of a single large dose of vitamin K_1 oxide) of the hypoprothrombinemia induced by repeated large doses of dicoumarol.

Case. No. U 99827. E.P., a white male, age 22 years, complained of lumbar pain associated with low grade fever and followed by a propagative right-sided femoral thrombophlebitis. Chart 1 shows the effects of treatment with heparin and dicoumarol.† Early in the

* Supported by a grant from the Columbia Foundation.

1 Chargaff, E., *J. Biol. Chem.*, 1938, **125**, 671.

2 Davidson, C. S., and MacDonald, H., *New Engl. J. Med.*, 1943, **229**, 353.

3 Campbell, H. A., Roberts, W. L., Smith, W. K., and Link, K. P., *J. Biol. Chem.*, 1940, **136**, 47.

4 Stahmann, M. A., Huebner, C. F., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 513.

5 Cahan, A., *New Engl. J. Med.*, 1943, **228**, 820.

6 Lucia, S. P., and Aggeler, P. M., *Clinics*, 1942, **1**, 414.

7 Davidson, C. S., and MacDonald, H., *Am. J. Med. Sci.*, 1943, **205**, 24.

8 Meyer, O. O., Bingham, J. B., and Axelrod, V. H., *Am. J. Med. Sci.*, 1942, **204**, 11.

9 Aggeler, P. M., and Lucia, S. P., *Acta Med. Scand.*, 1941, **107**, 179.

10 Bingham, J. B., Meyer, O. O., and Pohle, F. J., *Am. J. Med. Sci.*, 1941, **202**, 563.

† Supplied by E. R. Squibb and Company.

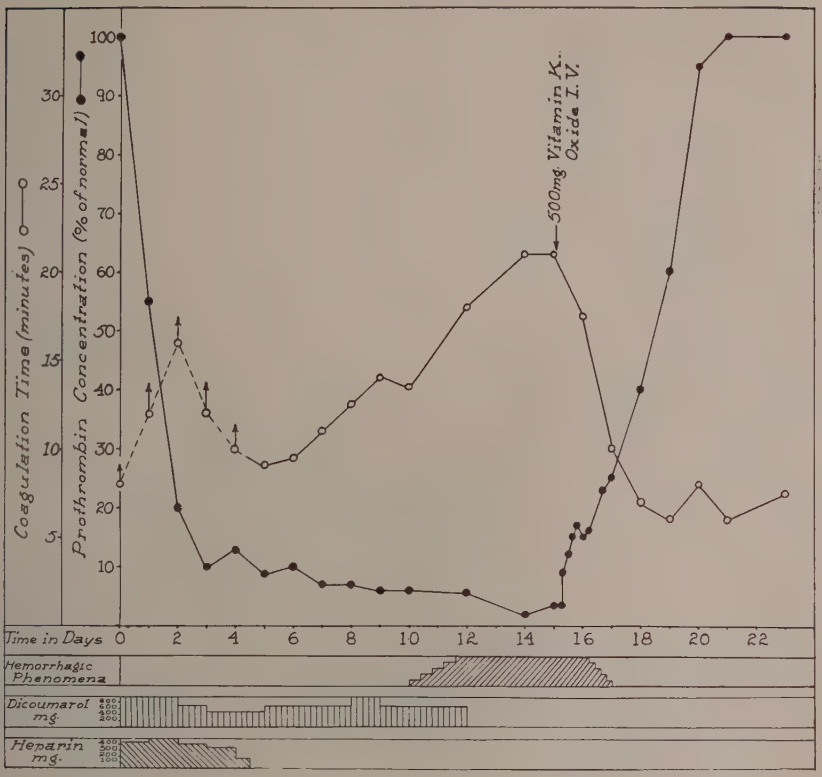

CHART I.

course of the illness, the whole blood coagulation time was retarded by intermittent intravenous doses of heparin,‡ while the effects of dicoumarol were being induced by the daily administration of 500 to 1000 mg oral doses of the drug.§ The prothrombin concentration dropped readily and was maintained at low levels for several days before significant alterations in the whole blood coagulation time occurred. Hematuria developed on the tenth day of dicoumarol medication and continued until the seventeenth day. During this time the patient had frequent epistaxis, ecchymoses at the site of hypodermic injections and bleeding from herpetic lesions on the lips. Dicoumarol treatment was discontinued on the twelfth day and on the fifteenth day 500 mg of vitamin K_1 oxide were given intravenously according to the method described by Davidson and MacDonald.[7] After a latent period of 4 hours, there was a dramatic elevation of the prothrombin level, although recovery from the hemorrhagic manifestations was delayed 24 hours. Full correction of the hypoprothrombinemia was achieved within 5 days. The thrombophlebitic process rapidly regressed after treatment with heparin and dicoumarol.

Summary. In a human subject, hypoprothrombinemia and the secondary hemorrhagic phenomena induced by multiple large doses of dicoumarol appeared to be corrected by the intravenous administration of a single massive dose of vitamin K_1 oxide. The period of time elapsing between the institution of treatment with vitamin K_1 oxide and the cessation of hemorrhagic phenomena is too long to make this method entirely satisfactory.

‡ 185 cc of Connaught Laboratories heparin was administered in 4½ days.

§ Total dosage of dicoumarol 9750 mg taken over a period of 12 days.

14584

Temperature and Blood Flow in Extremities Immersed in Water.*

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The present report contains data on the surface temperature of the hand immersed in water of various temperatures. The findings are discussed in relation to the blood flow through the hand.

Procedure. The subjects of these experiments sat in a controlled temperature room with one hand immersed in water until it was certain that thermal equilibrium had been established between the hand and the water as judged by skin temperature measurements. Observations were made at different room temperatures (16, 24, and 32°C) and at several water temperatures (5, 10, 15, 20, and 25°C).

Method. Skin temperatures of the naked hand immersed in water are difficult to evaluate. For this reason, the hand was encased in two rubber (surgeon's) gloves. Thermocouples (T_1) were placed in the medial aspect of the hand and on the lateral aspect of the first phalanx of the index finger. A second set of thermocouples (T_2) was placed directly over these after the first glove had been put on. And a third set (T_3) was similarly placed on the second rubber glove. A set of readings obtained at equilibrium over the finger under conditions where the difference between skin and water temperature was very great follows: T_1, 11.7; T_2, 8.7; T_3. 6.0; and T water, 5.0°C. From these readings it may be estimated that the true skin temperature is of the order of magnitude of 1°C above the value given by T_1. When skin and water temperatures are more nearly equal, the error in assuming T_1 to be the true skin temperature is correspondingly less. For the present purpose, no serious error is introduced if T_1 is regarded as the actual skin temperature.

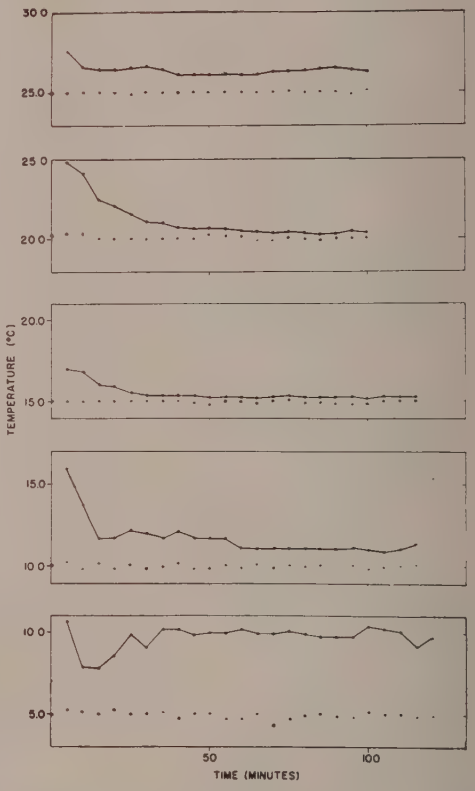

Fig. 1.

Finger skin temperature (solid lines) and water temperature (dots) plotted against time. These data were obtained on one subject who was studied at the 5 different water temperatures indicated. Room temperature was 16°C.

Results. Fig. 1, which shows a typical set of skin temperature measurements obtained on one subject, is given to illustrate the nature of the temperature data obtained. The minor fluctuations in skin temperature that occur in this subject are found with most subjects. In some they are more prominent. The figure also illustrates the fact that the difference between skin temperature and water tempera-

* The material in this article should be construed only as the personal opinion of the writer and not as representing the opinion of the Navy Department officially.

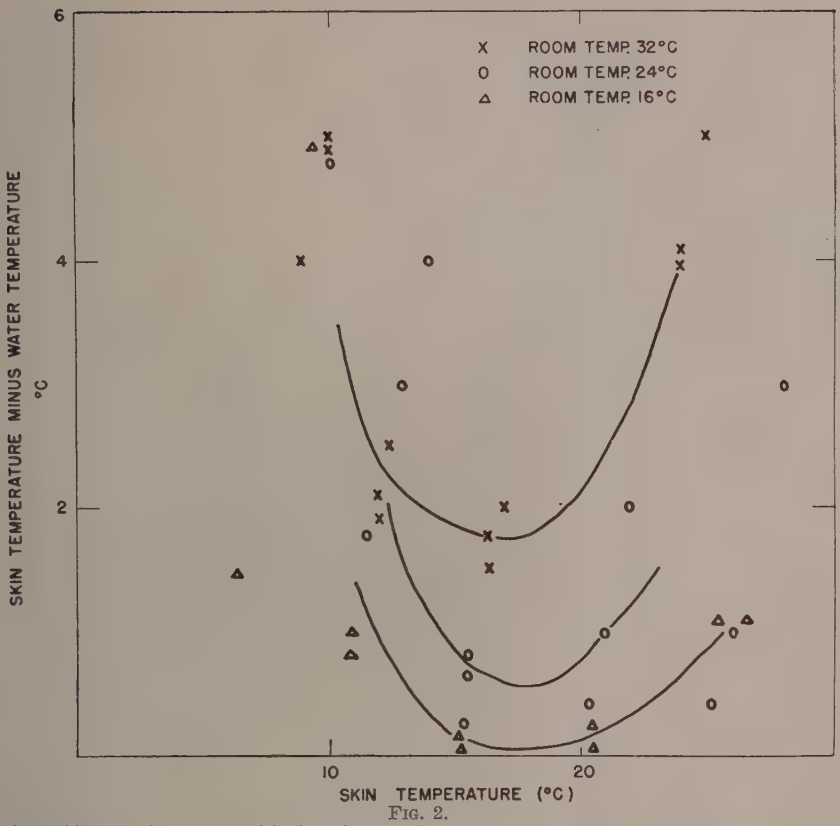

FIG. 2.

The difference between equilibrium finger skin temperature and water temperature, obtained from data such as are shown in Fig. 1, plotted against finger skin temperature. Data were obtained at room temperatures of 16°C (2 subjects), 24°C (3 subjects), and 32°C (3 subjects).

ture is least when the hand is immersed in moderately cold water.

In Fig. 2 the difference between skin and water temperature, obtained from data similar to those given in Fig. 1, is plotted against skin temperature. Data on 8 subjects studied at room temperatures of 16, 24, or 32°C are included. The figure illustrates the fact that the difference between skin temperature and water temperature becomes minimal at moderate temperatures. It also shows the effect of ambient air temperature. These data are for the finger; the data obtained on the hand are similar.

Discussion. The temperature difference between water and skin may be used as a measure of the comparative rates of heat loss from the hand at different water temperatures. For the subject of Fig. 1, these are 4.8, 0.9, 0.3, 0.3, and 1.1 for water temperatures of 5, 10, 15, 20, and 25°C respectively. Since equilibrium conditions exist, these are also the comparative rates of heat supply to the hand.

The rate of heat supply per unit volume of blood is proportional to the temperature difference between the blood entering the hand and the blood leaving it. The former may be taken to be 37°C. The temperature of the blood leaving the hand cannot differ greatly from the temperature of the hand surface, for the deep temperature of extremities immersed in water of a wide range of temperature has been shown not to differ greatly from

surface temperature. Barcroft[1] has recently reported on this.

The quantities of heat supplied per unit volume of blood are then roughly proportional to 27, 26, 22, 17, and 11 for water temperatures of 5, 10, 15, 20, 25°C respectively. Dividing the comparative figures for the rate of heat supply to the hand by the comparative figures for the heat supplied per unit volume of blood gives the following comparative figures for blood volume flow: 0.18, 0.03, 0.01, 0.02, and 0.10 for water temperatures of 5, 10, 15, 20, and 25°C respectively.

The above calculations involve assumptions used in the calorimetric method for determining blood volume flow, which has been criticized by Sheard.[2] In view of these criticisms, the above calculated figures should be regarded only as qualitative comparative blood flow values. Confirmatory of the relationship between blood flow and hand temperature inferred from these temperature data are results obtained using an adaptation of the plethysmographic technic of Hewlett and Van Zwaluwenburg.[3] The blood flow values found were 2.6, 2.6, 0.6, 0.5, and 1.4 cc per 100 cc of hand in one subject and 2.0, 1.1, 0.6, 0.4, and 0.8 cc per 100 cc of hand in a second subject for determinations in water at temperatures of 5, 10, 15, 20, and 25°C respectively. The above figures are the average of several determinations on each subject.

The comparative rates of blood flow inferred from the temperature data are in qualitative agreement with the results obtained with the conventional blood flow technic. Both results indicate that blood flow reaches low levels when the hand is immersed in water of 15 to 20°C and this is well known to be the case. In still colder water (10°C or below) the blood flow again increases. This fact does not seem to be generally realized, although Lewis[4] has presented evidence that vasodilation occurs in very cold water. Barcroft[1] and Freeman[5] have made studies on the blood flow in the arm and hand immersed in water of various temperatures; but neither apparently made studies at water temperatures sufficiently low to observe this effect. The lowest water temperature used by either was 13°C.

The data discussed above were obtained at a room temperature of 16°C. Other data on the temperature of the hand immersed in water were obtained at room temperatures of 24 and 32°C (Fig. 2). This figure shows that the difference between hand temperature and water temperature is generally increased as the room temperature is increased. It is, therefore, inferred that blood flow is likewise increased with increasing room temperature. This is in agreement with the findings of others, *e.g.*, Abramson *et al.*[6]

Summary. Skin temperature measurements made on the hand immersed in water of various temperatures show that the difference between skin and water temperatures is minimal when the hand is immersed in moderately cold water (15 to 20°C). With the higher (25°C) or lower (5 and 10°C) water temperatures studied, this difference is increased. The effect of increasing the temperature of the ambient air is generally to increase the difference between skin and water temperature; however, in very cold water, ambient air temperature seems to have less influence on this difference.

These results are discussed in terms of blood volume flow and the inference is made that blood flow through the hand passes through a minimum as the hand temperature is progressively lowered. This conclusion was verified using the plethysmographic technic of blood flow determination.

1 Barcroft, J., *J. Physiol.*, 1943, **102**, 5.

2 Sheard, Charles, *J. Clin. Invest.*, 1926, **3**, 327.

3 Hewlett, A. W., and Van Zwaluwenburg, J. G., *Heart*, 1909, **1**, 87.

4 Lewis, Thomas, *Heart*, 1929-1931, **15**, 177.

5 Freeman, N. E., *Am. J. Physiol.*, 1935, **113**, 384.

6 Abramson, D. I., and others, *Am. Heart J.*, 1939, **17**, 206.

14585

Leptazol and Diuresis.*

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Not uncommonly drugs have been introduced into medical practice for one purpose and subsequently found to be much more useful in other fields of therapy. This statement applies to the drug now known as Nikethamide in N.N.R. and the B.P., a drug which was originally marketed some 20 years ago under the name of Coramine and which, as the name implies, was at that time considered as a drug useful in the therapy of certain forms of cardiac disease. Nikethamide is now known to have little direct action upon the heart and blood vessels but in 1940 it was found in this laboratory that the drug is markedly diuretic if given in sufficiently large doses.[1] About the same time that Nikethamide was introduced. Leptazol, B.P.,[2] or Metrazol, N.N.R., was synthesized and marketed under the name of Cardiazol as a cardiac stimulant. Since leptazol is now known to have little direct action upon the heart and blood vessels,[3] it was decided to find out if it too might have diuretic properties.

Leptazol was injected subcutaneously into 6 groups, each of 21 healthy, adult albino rats averaging between 250 and 300 g body weight in doses of 0, 1, 5, 10, 25, and 50 mg per kilo body weight respectively. A dose of 100 mg per kilo body weight was found to be lethal to a majority of rats and was discarded early in the investigation. Up to the time of the experiment, the animals had had Purina Fox Chow Checkers and water *ad libitum*. They were then weighed, given the appropriate dose of leptazol or isotonic saline in a volume of 1 cc of isotonic saline per kilo body weight and placed in large funnels suitable for the collection of urine. At hourly intervals after injection, the animals were reweighed and the volume of urine noted.

Over the 6 hours during which the readings were taken, the average rate of urine formation was 34.4 cc per kilo per 24 hours and the rate of output was at no time affected by the injection of the several doses of leptazol, the output in the control group receiving injections of saline only being at the rate of 36.8 cc per kilo per 24 hours. These results were in marked contrast to those obtained with nikethamide in which the rate of urine formation was augmented to as much as 10 to 20 times the normal or control rate, as previously reported.[1]

Leptazol did increase the loss in total body weight. Most of this effect occurred during the first hour after injection of the drug and if the increased loss is expressed as a percent of the loss in total body weight of the controls given isotonic saline only, then it was calculated that for the 1 mg dose the increased loss averaged 7%, for the 5 mg dose 57%, for the 10 mg dose 100%, for the 25 mg dose 157%, and for the 50 mg dose 129%. By the end of 6 hours after injection, the cumulative loss of total body weight in the controls was similar to that in the rats receiving leptazol with the exception of the 25 and 50 mg doses of leptazol and rats receiving these large doses of leptazol still had a cumulative loss in total body weight which averaged 39% over that in the controls.

Conclusion. It was not possible to demonstrate that leptazol had a diuretic action in albino rats when given in doses up to the lethal dose.

* The authors wish to acknowledge with thanks the cooperation, financial and otherwise, of Dr. E. A. Bilhuber of the Bilhuber-Knoll Corporation.

1 Boyd, E. M., and Forde, J. D., *J. Pharm. and Exp. Therap.*, 1940, **70**, 279.

2 *Third Addendum to the British Pharmacopoeia*, 1932, Constable and Co., London, 1941.

3 Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, Macmillan, New York, 1941.

14586

Studies on Sulfonamide-Resistant Organisms. IV. Retention of Resistance by Pneumococci.

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It is now a well established fact that under certain conditions bacteria develop resistance to the sulfonamides. If this resistance should be a permanent characteristic and these non-susceptible organisms become widely disseminated by passage from one individual to another, sulfonamide therapy in future years may be much less successful than at present. For this reason it is important to know whether organisms which have become resistant to the sulfonamides retain this characteristic indefinitely after being removed from the influence of these drugs.

Previously reported experiments[1] showed that each of 4 strains of pneumococcus, made resistant to the sulfonamides, retained this property for at least 1 year. During this time these strains underwent approximately 200 passages through mice and an equal number in infusion broth without any contact with a sulfonamide. Since the former report, these strains have undergone similar passages for an additional 2 years. A fifth strain of pneumococcus, made resistant in another laboratory, has also been observed for a period of almost 3 years.

Experimental. Four of the strains used in this study were made resistant in this laboratory. The type I McGovern, type III CHA, and type III Wistuba strains were made resistant to sulfapyridine by serial passage through groups of mice treated with gradually increasing doses of this drug. The type II CH strain was made resistant by serial passage through broth containing increasing sulfapyridine concentrations. Details of this work have been described elsewhere.[1] The fifth strain, type I P47,* was also made resistant by serial passage through media containing sulfapyridine.[2] As originally prepared, all 5 strains were resistant both *in vivo* and *in vitro*.

These resistant strains and their sensitive parent strains were passed alternately through untreated mice and through beef infusion broth enriched with rabbit blood, either daily or every other day for approximately 3 years. At various intervals, the strains were tested for *in vivo* and *in vitro* responses to sulfapyridine.

In most of the *in vivo* tests, groups of 30 to 40 white mice (males weighing 18 to 22 g) were infected intraperitoneally. The infecting dose was 1 cc of a 10^{-6} dilution in infusion broth of a 12- to 14-hour blood broth culture obtained from the heart blood of a passage mouse. Part of the mice served as untreated controls; the remainder were treated by stomach tube with 20 mg doses of sulfapyridine. These doses, suspended in 10% gum acacia, were administered 2, 8, and 14 hours after infection and every 8 hours thereafter for 5 additional days or as long up to that time as the animals survived.

The results with 4 of the resistant strains and their sensitive parent strains are presented in Table I. Although determinations of drug sensitivity were made at approximately 6-month intervals, only the first and latest determinations are shown here, inasmuch as the results of intervening experiments were not significantly different from those described. It is evident from the data that these 4 resistant strains had essentially the same response to sulfapyridine at the end of the 3-year period as when made resistant. With reference to the corresponding parent strains, 3 showed no significant change in response to the above sulfonamide; the fourth strain (type II CH) was somewhat less sensitive at the end of 3 years passages than originally, but

[1] Schmidt, L. H., Sesler, C. L., and Dettwiler, H. A., *J. Pharm. and Exp. Therap.*, 1942, **74**, 175.

* This strain and its sensitive counterpart SV-1 were obtained from Dr. C. M. MacLeod.

[2] MacLeod, C. M., and Daddi, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

TABLE I.
Retention of Sulfonamide Resistance by Type I McGovern, Type II CH, Type III CHA, and Type III Wistuba Strains of Pneumococcus.

Organism	Date of test	No. of mouse passages*	No. of infecting organisms	Treatment†	No. of deaths Days after infection 1	2	3	4	5	6	7-30	Avg hrs survival of mice that died	30-day survivors No.	%
Type I McGovern														
Resistant strain	5/21/40	14	1800	SP	0	22	8	0	0	0	0	47	0	0
				O	4	6	0	0	0	0	0	26	0	0
	7/28/43	605	810	SP	0	3	12	5	0	0	0	54	0	0
				O	3	7	0	0	0	0	0	26	0	0
Parent strain	6/ 7/40	22	1760	SP	0	0	0	0	0	1	2	148	27	90
				O	3	7	0	0	0	0	0	27	0	0
	7/28/43	605	560	SP	0	0	0	0	0	1	7	267	12	60
				O	2	8	0	0	0	0	0	27	0	0
Type II CH														
Resistant strain	5/21/40	12	1510	SP	0	29	1	0	0	0	0	38	0	0
				O	1	9	0	0	0	0	0	27	0	0
	9/27/43	610	290	SP	0	20	0	0	0	0	0	35	0	0
				O	3	7	0	0	0	0	0	25	0	0
Parent strain	8/18/40	60	250	SP	0	0	0	0	0	0	19	184	11	37
				O	0	10	0	0	0	0	0	30	0	0
	9/27/43	610	340	SP	0	0	0	0	0	1	19	172	0	0
				O	6	4	0	0	0	0	0	24	0	0
Type III CHA														
Resistant strain	8/ 9/40	1	300	SP	0	19	10	1	0	0	0	44	0	0
				O	7	3	0	0	0	0	0	24	0	0
	9/20/43	550	210	SP	0	19	0	0	0	0	0	36	1	5
				O	5	5	0	0	0	0	0	26	0	0
Parent strain	8/ 9/40	1	300	SP	0	0	0	0	1	0	29	186	0	0
				O	6	4	0	0	0	0	0	24	0	0
	9/20/43	550	570	SP	0	0	0	0	0	0	19	173	1	5
				O	4	6	0	0	0	0	0	27	0	0
Type III Wistuba														
Resistant strain	11/24/40	1	800	SP	0	21	9	0	0	0	0	44	0	0
				O	6	4	0	0	0	0	0	25	0	0
	9/20/43	460	500	SP	0	20	0	0	0	0	0	34	0	0
				O	9	1	0	0	0	0	0	22	0	0
Parent strain	10/28/40	‡	500	SP	0	0	0	0	0	1	15	173	4	20
				O	7	3	0	0	0	0	0	25	0	0
	9/20/43	460	550	SP	0	0	0	0	0	2	17	171	1	5
				O	4	6	0	0	0	0	0	25	0	0

* Mouse passages since preparation of the resistant strain.

† Treatment consisted of 20 mg doses of sulfapyridine, administered 2, 8 and 14 hours after infection, and every 8 hours thereafter for 5 additional days. O indicates no treatment.

‡ Wistuba parent strain not tested on 11/24/40; test of 10/28/40 nearest to above date.

was not at all like its resistant counterpart.

In contrast to the above findings, the fifth strain of pneumococcus, type I P47, lost much if not all of its resistance to sulfapyridine (Table II). There is a suggestion that this change occurred gradually, since the percentage of surviving mice increased progressively. Data on this point are not entirely conclusive, for only small numbers of mice were used in several of the intermediate experiments. It is well to note that the loss in resistance was not related to a loss of virulence; actually, the P47 strain had higher virulence at the end of 3 years than when originally received. It should also be noted that strain SV-1, the sensitive parent of P47, did not change its response to sulfapyridine during the period of observation.

The *in vitro* response of the various strains to sulfapyridine was determined at approximately the same times as the *in vivo* response. The technics of the *in vitro* tests have been detailed elsewhere.[3] The results of the tests, shown in Table III, confirm the *in vivo* find-

[3] Schmidt, L. H., Ruegsegger, J. M., Sesler, C. L., and Hamburger, M., Jr., *J. Pharm. and Exp. Therap.*, 1941, **73**, 468.

TABLE II.
Loss of Sulfonamide Resistance *in Vivo* by a Moderately Resistant Strain of Pneumococcus.

Organisms	Date of test	No. of mouse passages*	No. of infecting organisms	Treatment†	No. of mice infected	No. of deaths Days after infection 1	2	3	4	5	6	7-30	Avg hrs survival of mice that died	30-day survivors No.	%
McLeod, type I															
P47, Resistant	1/8/41	40	500	SP	30	0	0	7	9	5	0	9	113	0	0
				O	10	0	9	1	0	0	0	0	40	0	0
	1/28	60	900	SP	30	0	0	7	14	2	2	1	86	4	13
				O	10	0	9	0	0	0	0	0	41	1	10
	6/14	130	260	SP	30	0	0	0	8	3	5	12	139	2	7
				O	10	1	7	2	0	0	0	0	42	0	0
	1/15/42	225	478	SP	10	0	0	0	4	0	0	3	124	3	30
				O	5	0	5	0	0	0	0	0	28	0	0
	9/10	325	140	SP	10	0	0	0	0	0	0	0	—	10	100
				O	5	0	5	0	0	0	0	0	34	0	0
	9/27/43	470	340	SP	20	0	0	0	0	1	0	3	267	16	80
				O	10	0	10	0	0	0	0	0	32	0	0
SV-1, Parent	1/8/41	40	350	SP	30	0	0	0	0	0	0	1	—	29	97
				O	10	0	5	3	0	0	0	1	67	1	10
	1/28	60	580	SP	29	0	0	0	0	0	0	0	—	29	100
				O	10	0	9	0	0	1	0	0	50	0	0
	1/15/42	225	470	SP	10	0	0	0	0	0	0	1	—	9	90
				O	5	0	5	0	0	0	0	0	39	0	0
	10/12	325	850	SP	10	0	0	0	0	0	0	0	—	10	100
				O	5	0	5	0	0	0	0	0	39	0	0
	9/27/43	470	410	SP	20	0	0	0	0	0	0	1	—	19	95
				O	10	0	10	0	0	0	0	0	32	0	0

* Number of mouse passages after these strains were received from Dr. C. M. MacLeod.
† Treatment consisted of 20 mg doses of sulfapyridine, administered 2, 8 and 14 hours after infection, and every 8 hours thereafter for 5 additional days; O indicates no treatment.

ings reported above. An *in vivo*, the resistant McGovern, CH, CHA, and Wistuba strains showed no significant changes in response to sulfapyridine throughout the 3-year period. Likewise, the parent McGovern, CH, CHA, and Wistuba strains did not change in their reactions.

As in the *in vivo* experiments, the P47 strain lost its resistance *in vitro*. In the first experiment this strain grew in 10 mg % sulfapyridine.† Nine months later and at all times thereafter, the P47 strain was unable to grow in more than 2.5 mg % sulfapyridine. The data suggest that loss of resistance *in vitro* preceded the loss *in vivo*. The sensitive parent strain did not change in sulfapyridine sensitivity throughout the 3-year period.

Comment. The above data would seem to indicate that in some instances acquired sulfonamide resistance is a permanent characteristic, whereas in other cases it is merely a transitory property. It is noteworthy that the 4 resistant strains of pneumococcus which showed no change in sulfonamide response were highly resistant at the start of this study. On the other hand, the fifth strain, which became more responsive, was only moderately resistant at the outset. This loss of resistance by pneumococci which have acquired only moderate resistance has been observed previously in both experimental[4] and clinical[5] studies.

Previous work[6] on the development of sulfonamide resistance in pneumococci suggests

† According to the data reported by MacLeod,[2] the P47 strain grew in 1/16,000 sulfapyridine (6.2 mg %) in serum broth as compared with 1/160,000 (0.6 mg %) for the SV-1 parent strain. The differences in inhibiting concentrations found in his work and those found in this laboratory may be due to differences in experimental technics.

[4] Dettwiler, H. A., and Schmidt, L. H., *J. Biol. Chem.*, 1940, **133**, lxxxv.

[5] Hamburger, M., Jr., Schmidt, L. H., Sesler, C. L., Ruegsegger, J. M., and Grupen, E. S., *J. Inf. Dis.*, 1943, **73**, 12.

[6] Schmidt, L. H., and Sesler, C. L., *J. Pharm. and Exp. Therap.*, 1943, **77**, 165.

TABLE III.
Retention or Loss of Sulfonamide Resistance *in Vitro* by 5 Strains of Pneumococcus.

Organism	Date of test	Highest concentration of sulfapyridine permitting visible growth in 24 hr	
		Resistant strain mg %	Parent strain mg %
McGovern, type I	1/14/41	20	<5*
	9/28/43	20	5
CH, type II	1/10/41	80	<5*
	9/28/43	80	2.5
CHA, type III	1/20/41	40	5
	9/28/43	80	2.5
Wistuba, type III	1/23/41	40	5
	9/28/43	40	2.5
P47, SV-1, type I	1/ 8/41	10	<5*
	10/16/41	<2.5*	0.6
	3/ 5/42	2.5	<0.6*
	8/12/43	2.5	1.25

* Lowest sulfapyridine concentration used in this test.

that the retention or loss of resistance probably depends on the homogeneity of the organisms composing a particular resistant strain. This earlier work showed that development of a resistant strain involves a stepwise formation of pneumococci of increasing resistance, with selective propagation of the more resistant organisms in the culture and consequent elimination of the more sensitive bacteria. Thus the more resistant a strain becomes, the less likely it is to contain sensitive organisms. Such highly resistant strains as McGovern, CH, CHA, and Wistuba are probably composed solely of highly resistant organisms. On the other hand, moderately resistant strains like P47 and our earlier CHA[4] probably contained both moderately resistant and sensitive organisms. These latter strains may in time lose resistance if the particular sensitive organisms are better able to grow in the mouse or artificial culture medium than are the moderately resistant organisms.

The practical implications of these observations on the retention of sulfonamide resistance are obvious. Dissemination of highly resistant organisms would probably be a considerable hazard; dissemination of moderately resistant organisms would be a much less serious problem.

Conclusions. Strains of pneumococcus which have acquired a high degree of resistance to the sulfonamides retain that resistance for an indefinite period. Strains which have acquired only a moderate degree of resistance may lose this characteristic after removal from contact with a sulfonamide.

14587

Adaptation of Virus of Epidemic Keratoconjunctivitis to Development in Extra-Embryonic Fluids of Chick Embryo.

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Recent discussion[1,2,3] of the size and nature of influenza virus has been based upon the properties of strains which have been adapted to the extra-embryonic fluids of the chick embryo.

This note is presented in the belief that it is important for other viruses to be adapted to a similar technic so as to be available for similar study.

The strain of epidemic keratoconjunctivitis virus[4] used in this investigation, was isolated by tissue culture and established in mouse brain by Major M. Sanders, MC, AUS. The original material received from Major Sanders was given 2 mouse brain passages, and approximately 10% suspensions of whole macerated brain from the last passage were inoculated into the chorio-allantoic fluid of 8-day chick embryos. The embryos died in 5 or 6 days, the chorio-allantoic and amniotic fluids were collected, tested for sterility, and pooled.

After 2 more embryo-fluid passages, the fluid from the third passage was reinoculated into young mice. The mouse brain material was again passed three times in embryo fluids and returned to mouse brain.

This last mouse passage material has been maintained in 12 consecutive embryo-fluid passages, with increase in virulence so that the present material is lethal in chick embryos in 0.001 ml quantities, killing them in 3 or 4 days. The material still produces a fatal encephalitis in mice.

Human convalescent and hyperimmune rabbit sera, furnished us by Major Sanders, will neutralize the embryo-fluid passage strain so that it will not cause the death of chick embryos. (Table I.)

The ease of handling this virus in extra-embryonic fluids, and the success of the neutralization tests, suggest that this method might be of value in serological diagnostic work. Investigations along this line are now in progress.

TABLE I.
Neutralization of Keratoconjunctivitis Virus in Chick Embryos.

Tube	*Virus, cc	Antiserum	cc	Diluent, cc	8-day inoculated embryos	Embryos dead in 8 days
1	0.1	Human convalescent	0.3	4.6	12	0
2	0.1	Hyperimmune rabbit	0.3	4.6	12	1
3	0.1	Normal sheep serum	0.3	4.6	12	12
4	0.1	—		4.9	12	11

* Chorio-allantoic fluid—10th passage.

1 Chambers, L. A., *et al.*, *J. Exp. Med.*, 1943, **77**, 265.

2 Taylor, A. R., *et al.*, *J. Immunol.*, 1943, **47**, 261.

3 Stanley, W. M., *J. Exp. Med.*, 1944, **79**, 267.

4 Sanders, M., and Alexander, R. C., *J. Exp. Med.*, 1943, **77**, 71.

14588

Saprophytic Acidfast Bacilli and Paraffin Oil as Adjuvants in Immunization.*

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It was found recently[1] that horse serum combined with a lanolin-like substance and killed tubercle bacilli suspended in paraffin oil induces more vigorous antibody formation and sensitization than horse serum alone. Antibody formation and sensitization last for a remarkably long time in the animals injected with horse serum and the adjuvants.

The use of these potentiating agents was applied by Landsteiner and Chase[2] and Chase[3] to sensitization and antibody formation using chemicals and protein conjugates. Kopeloff and Kopeloff[4] with the aid of this method succeeded in demonstrating antibody formation against brain extracts in the *macacus rhesus* monkey.

The purpose of the present study was to determine whether *Mycobacterium phlei* can be substituted for tubercle bacilli and what the effect of the lanolin-like substance and paraffin oil is on antibody formation and sensitization. It seemed important to ascertain whether killed timothy bacilli under the conditions of the experiment induce sensitization to tuberculin.

Four groups of guinea pigs weighing from 590 to 1320 g received in the subcutaneous tissue of the back of the neck 0.5 ml of antigen diluted with salt solution or combined with the adjuvants. The materials used are shown in Table I.

The intervals between the injection of antigen and the tests were as follows: titration of antibodies against horse serum 47, test for skin sensitivity to horse serum 49, to tuberculin 62, and to phleolin 133 days.

The collodion agglutination test was done by coating collodion particles with a 1:10 dilution of horse serum. The washed particles were then suspended in 1 ml of guinea pig serum dilutions, the mixtures kept at 4°C overnight and read the next day. Details of the technic may be found in previous publications.[5]

Sensitization to horse serum was tested by intracutaneous injections of 0.1 ml horse serum, either undiluted or diluted 1 in 10. Tuberculin and phleolin tests were made by injecting 0.1 ml of diluted material into the skin.

Table II shows that horse serum in combination with Falba and paraffin oil induced antibody formation more effectively than horse serum alone; the addition of killed acidfast bacilli to the water-in-oil emulsion had an additional augmenting effect on the production of antibodies. Tubercle bacilli were slightly more effective than timothy bacilli.

Table III shows the extent of skin reactions to undiluted and diluted horse serum. Falba and oil seem to have enhanced sensitization only very slightly if at all but the addition of acidfast bacilli to the emulsion intensified the sensitization in a striking manner. Again tubercle bacilli were more effective than timothy bacilli.

* This study was aided by a grant from the John and Mary R. Markle Foundation.

1 Freund, J., and McDermott, K., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 548.

2 Landsteiner, K., and Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 688.

3 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 238.

4 Kopeloff, L. M., Barrera, S. E., and Kopeloff, N., *Am. J. Psychiat.*, 1942, **98**, 881; Kopeloff, L. M., and Kopeloff, N., *Fed. Proc.*, (Am. Soc. for Exp. Biol.), 1943, **2**, 99; Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1944, **48**, 297.

5 Freund, J., PROC. SOC EXP. BIOL. AND MED., 1930, **28**, 6570; *J. Exp. Med.*, 1932, **55**, 181; *Science*, 1932, **75**, 418.

TABLE I.
Material Injected.

Group	Horse serum, ml	Falba,* ml	Paraffin oil, ml	Killed tubercle bacilli, mg (dry wt)	Killed timothy bacilli, mg (dry wt)	Salt sol. 0.85% ml
A	.125	.125	.25	.1	0	0
B	.125	.125	.25	0	.1	0
C	.125	.125	.25	0	0	0
D	.125	0	0	0	0	.375

* Falba is the trade name of a lanolin-like substance prepared by Pfaltz and Bauer, Inc., New York City.

TABLE II.
Collodion Agglutination Titers.
Number of Sera with Titers Indicated.

	Serum dilutions								
Group	640	320	160	80	40	20	10	5	<5
A	2	3	3						
B		2	2	3					1
C	1		2	1	1	1	2		
D						1	3	2	1

Sera from unimmunized guinea pigs did not react in dilutions higher than 1:5.

TABLE III.
Skin Reactions to Horse Serum.
Number of Guinea Pigs with Reactions Designated.

		Horse serum													
		Undiluted								Diluted 1:10					
Reading	Group	N	P	5	4	3	2	1	±	N	3	2	1	±	0
24 hr	A	3	5	2	5	1				2	1	7			
	B		3		6	2					2	1	3	2	
	C		2	1	3	1	3						1		7
	D				4	1	1							2	4
48 "	A	8		4	4					1			4	1	3
	B	1		4	3	1							1	2	5
	C	1		1	5	1		1							8
	D				2	3	1								6
72 "	A	8		4	1	2		1		3			2	3	3
	B	5		4	1	2	1			1			1	1	5
	C	1			1	3	2	1	1					1	7
	D				2	3	1								6

N Necrosis.
P Purple area.
Edema—diameter in mm:
5 75 or more
4 60-74
3 45-59
2 30-44
1 15-29
± Doubtful
0 Less than 15

Table IV shows that all of the guinea pigs which received killed tubercle bacilli became highly sensitive to tuberculin but only one of them reacted to phleolin. Seven of 8 animals injected with timothy bacilli developed sensitiveness to phleolin and all of them reacted to

TABLE IV.
Skin Reactions to Tuberculin and Phleolin.
Number of Guinea Pigs with Reactions Designated.

	Tuberculin						Phleolin					
	1:100			1:1000			1:100			1:1000		
Group	N	3	2	2	1	0	2	1	0	2	1	0
A	3	6	2	8				1	7			8
B			8		1	7	7		1	3	1	4

Reactions read 48 hours after test injections:
N Necrosis.
Induration—diameter in mm:
3 20-29
2 10-19
1 5-9
0 Less than 5

tuberculin. It may be added that the animals injected with horse serum incorporated in Falba and oil did not react to either tuberculin or phleolin.

At the site of the injections of mixtures containing tubercle or timothy bacilli palpation did not reveal the presence of nodes and suppuration through the skin did not occur.

Discussion. In a previous study[6] it was found that the incorporation of killed typhoid bacilli suspended in salt solution into lanolin-like substances (Aquaphor or Falba) and paraffin oil enhances and prolongs the antigenic effect of typhoid bacilli as judged by the formation of agglutinins. The data presented above concerning antibody formation against horse serum as measured by the collodion agglutination test are in harmony with our previous observations. It is interesting to note that Falba and paraffin oil alone had little if any effect on sensitization to horse serum.

In the preceding paper[6] observations were made as to the effect of the addition of killed tubercle bacilli to a water-in-oil emulsion containing typhoid bacilli. It was found that tubercle bacilli had an additional potentiating effect. In the present experiment a similar result was seen in relation to antibody formation against horse serum. Furthermore it was found that the timothy bacilli employed in this experiment were almost as effective as the pathogenic acidfast bacilli.

[6] Freund, J., and Bonanto, M. V., *J. Immunol.*, 1944, **48**, 325.

Not only tubercle bacilli but also timothy bacilli sensitized the guinea pigs to tuberculin although to a lesser degree. Sensitization to phleolin, however, was demonstrable in only 1 of 8 animals receiving tubercle bacilli; 7 of 8 pigs injected with timothy bacilli reacted to phleolin. It is possible that more of the guinea pigs with tubercle bacilli might have reacted to a higher concentration of phleolin than the one used in this experiment. It may be added that the potency of tuberculin and that of phleolin can not be compared. From the standpoint of human immunization cross-sensitization is important, particularly the observation that timothy bacilli sensitized to tuberculin. The synergistic agents studied may be applicable to the immunization of experimental and domestic animals. In man killed tubercle or even timothy bacilli when suspended in paraffin oil may cause suppuration particularly in persons sensitive to tuberculin. Search is in progress to find potentiating agents to replace the acidfast bacilli employed in the present experiment.

Paraffin oil may cause persistent nodules. Vegetable oil might be less objectionable in immunization of man. It was shown in a preceding paper[6] that peanut oil is effective though not as much so as paraffin oil in enhancing and sustaining antibody formation. A comparative study of various vegetable oils is in progress.

Conclusions. 1. A lanolin-like substance and paraffin oil when combined with horse serum enhance the formation of antibodies but have little if any effect on sensitization to

horse serum. 2. Killed timothy bacilli added to the water-in-oil emulsion promote both antibody formation and sensitization almost as effectively as killed tubercle bacilli. 3. Under the conditions of this experiment killed timothy bacilli sensitize to tuberculin.

14589

Agglutination of Circulating Leukocytes by Antileukocytic Sera.

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Several investigators had produced antisera against animal and human leukocytes,[1-6] but no successful attempt has been made to test and titrate the antileukocytic serum by agglutination. Some of the workers stated that the cytotoxic qualities of serum prevent a correct evaluation by agglutination.[2,4,7,8] Hueper and Russell[4] expressed the view that tissue culture is the only satisfactory method for correct titration of antileukocytic serum. In our experiments herein reported we were more successful and are able to present a method of agglutinating circulatory leukocytes by antileukocytic sera and of titrating the antisera.

Leukocytic agglutinins were produced in Chinchilla rabbits by intravenous injections of leukocytes secured from normal and leukemic individuals. Normal leukocytes were obtained from blood bank material used for preparation of plasma. The blood was centrifuged, the buff layer removed and washed several times. The animals received a total of 12 to 14 injections of 11,700,000 to 20,100,000 cells suspended in one cc of normal salt solution, twice weekly. Testing was done on the serum pooled from at least 3 animals. Blood was obtained by cardiac puncture in volumes of 10 to 15 cc. When agglutinins were demonstrated in a dilution of 1:2560 the antiserum was considered satisfactory for titration. Antisera were developed against lymphocytes and against cells of the granulocytic series of mature and immature types by the use of blood from normal individuals and from those with lymphoid and myeloid leukemia. Normal rabbit serum and normal salt solution were used as controls for the antisera.

Titrations were set up in a series of 10 x 75 mm test tubes. Blood was collected and mixed with a dry anticoagulant of ammonium and potassium oxalate, centrifuged and the buff layer pipetted off. The leukocytes were suspended in and the antisera diluted with normal salt solution. The necessity for the presence of electrolytes for the visible phase of agglutination has been demonstrated.[9,10] To ascertain the optimum number of leukocytes for an agglutination test, the cell concentration was varied and the volume of antiserum was kept constant. It was determined that 0.05 cc of normal salt solution containing 27,000 cells per cu mm constituted a satisfactory suspension. The leukocyte suspension was added to 0.5 cc volumes of inactivated antiserum diluted with normal salt solution in dilutions of from

[1] Ledingham, J. C. G., and Bedson, S. P., *Lancet*, 1915, **1**, 311.

[2] Lindström, G. A., *Acta Med. Scandinav.*, Supp., 1927, 22.

[3] Matsumo, M., *Tohoku J. Exp. Med.*, 1932, **19**, 168.

[4] Hueper, W. C., and Russell, M. A., *Arch. Path.*, 1932, **13**, 584.

[5] Chew, W. B., Stephens, D. J., and Lawrence, J. S., *J. Immunol.*, 1936, **30**, 301.

[6] Chew, W. B., and Lawrence, J. S., *J. Immunol.*, 1937, **33**, 271.

[7] Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, **14**, 453.

[8] Foot, N. C., *Centralbl. f. allg. Path. u. path. Anat.*, 1912, **23**, 578.

[9] Bordet, J., *Traité de l'Immunité*, Paris, 1920.

[10] Marrack, J. R., *The Chemistry of Antigens and Antibodies*, Med. Res. Council, Spec. Rep. Ser. 194, London, 1934.

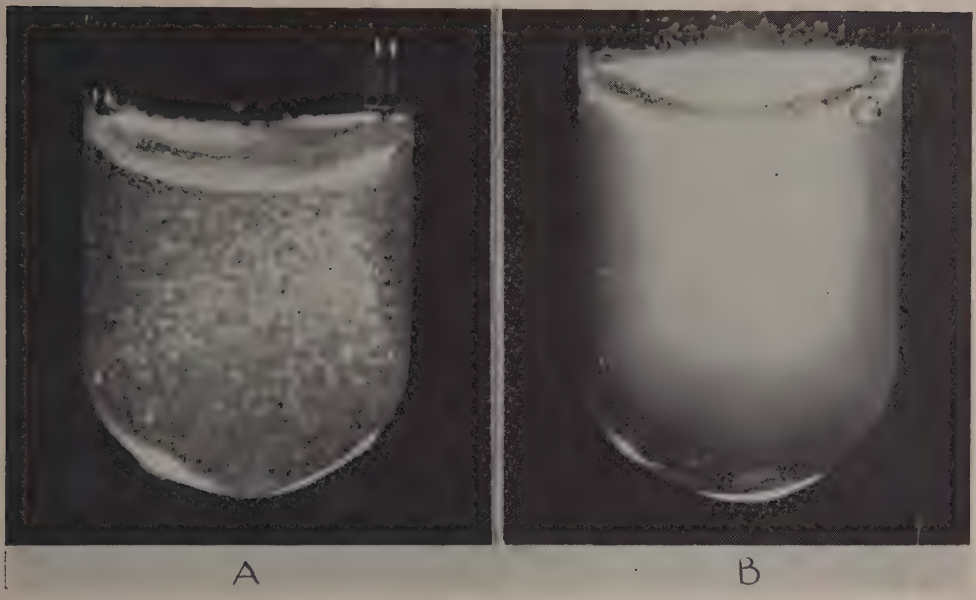

FIG. 1.
Gross appearance of agglutination of blood leukocytes by anti-leukocytic serum in a dilution of 1:2560. A. Positive reaction. B. Negative reaction.

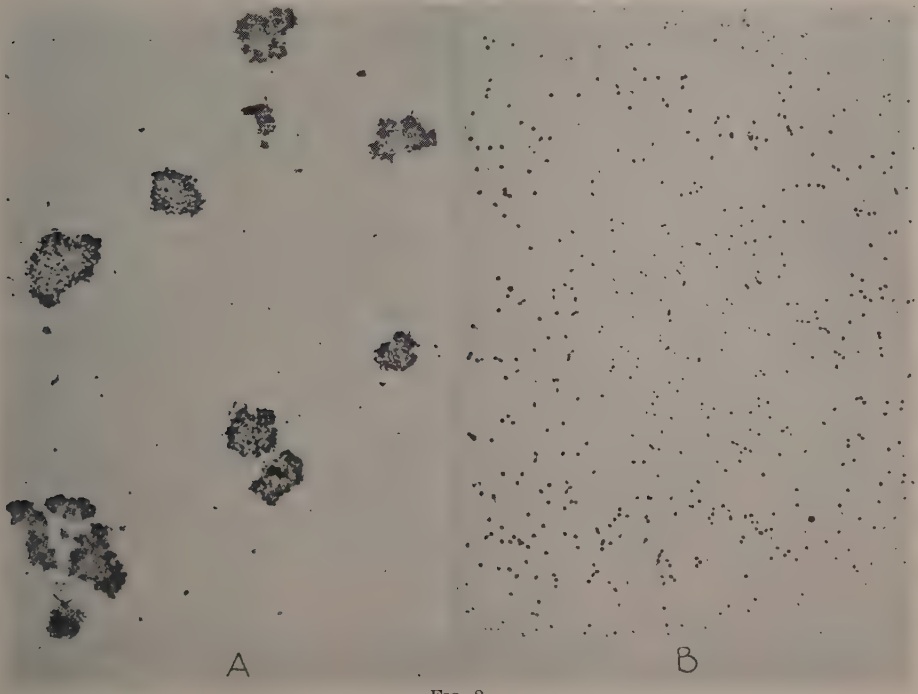

FIG. 2.
Microscopic appearance of agglutination of blood leukocytes by anti-leukocytic serum in a dilution of 1:2560. A. Positive reaction. B. Negative reaction.

1:10 to 1:5120. After shaking the test tubes for 3 minutes, they were placed in a water bath at 37°C for one hour. At the end of that time the tubes were shaken again and placed in the refrigerator over night.

Readings were made in the morning. After gentle agitation of the test tubes, presence of cell aggregates was determined first grossly under proper lighting conditions. A negative reaction was seen as a diffuse cloudiness. Agglutination varied in intensity depending on the original titer of the antiserum and the dilution. A strong reaction consisted in the presence of large clumps. As the agglutinin titer of the antiserum decreased, the cell aggregates became smaller and fewer.

Microscopic readings were made by pipetting a drop of the leukocyte-antiserum mixture onto a slide and examining it with a 16 mm objective. Clumping of the leukocytes with loss of identity of the individual cell constituted a positive reaction. Partial or complete disintegration of the cell architecture could be seen in the stronger reactions. In many instances the "prozone" phenomenon was encountered.

The technic of the test is not overly simple. The natural tendency of leukocytes, especially from normal people, to clump tends to produce false agglutination. After some experience, such reactions can be recognized grossly. In most instances of false agglutination, the leukocytes can be observed under the microscope to be held together loosely by strands of fibrin.

Summary. A method of testing and titrating antileukocytic sera by agglutination is presented. The procedure offers a relatively simple method of studying the immunochemical differences between normal and leukemic leukocytes and between lymphocytes and polymorphonuclear leukocytes.

14590

The Static Intrapelvic Pressure of the Hydronephrotic Kidney.

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Following complete ureteral obstruction the hydronephrotic kidney continues to secrete urine. The exact quantity of the glomerular filtrate elaborated and the mode of reabsorption is not known with certainty. The reabsorption of urine by pyelovenous refluxes or pyelotubular backflow is a possibility.[1,2,3] Ahlström[4] points out that with an increased intrapelvic pressure, rupture of the pelvis with an outpouring of its contents into the venous system is not, in itself, remarkable. Only when this reflux can be shown to occur at pressures which are maintained *in vitro* by urine secretion with obstruction to outflow can it be of pathogenic importance.[4] Despite the crucial importance of intrapelvic pressures, accurate determinations by physiologic methods have not been found in the literature, by the writer.

Experimental. By the use of the Null Point Manometer which is on occasion employed in various physiological laboratories we have determined the static intrapelvic pressure in 8 rabbits with unilateral hydronephrosis of varying intervals from 3 to 63 days. The left ureter of the rabbits was doubly tied and severed; intravenous sodium pentothal anesthesia, and aseptic surgical precautions were employed. The needle attached to the left arm of the Null Point Manometer System is inserted into the hydronephrotic sac through the thin wing of parenchyma near the edge

[1] Hinman, F., and Lee-Brown, R. K., *J. Am. Med. Assn.*, 1924, **82**, 607.

[2] Hinman, F., and Vecki, M., *J. Urol.*, 1926, **15**, 267.

[3] Hinman, F., *Surg., Gynec. and Obstet.*, 1934, **58**, 356.

[4] Ahlström, C. G., *Acta chir. Scandinav.*, 1934, **75**, 162.

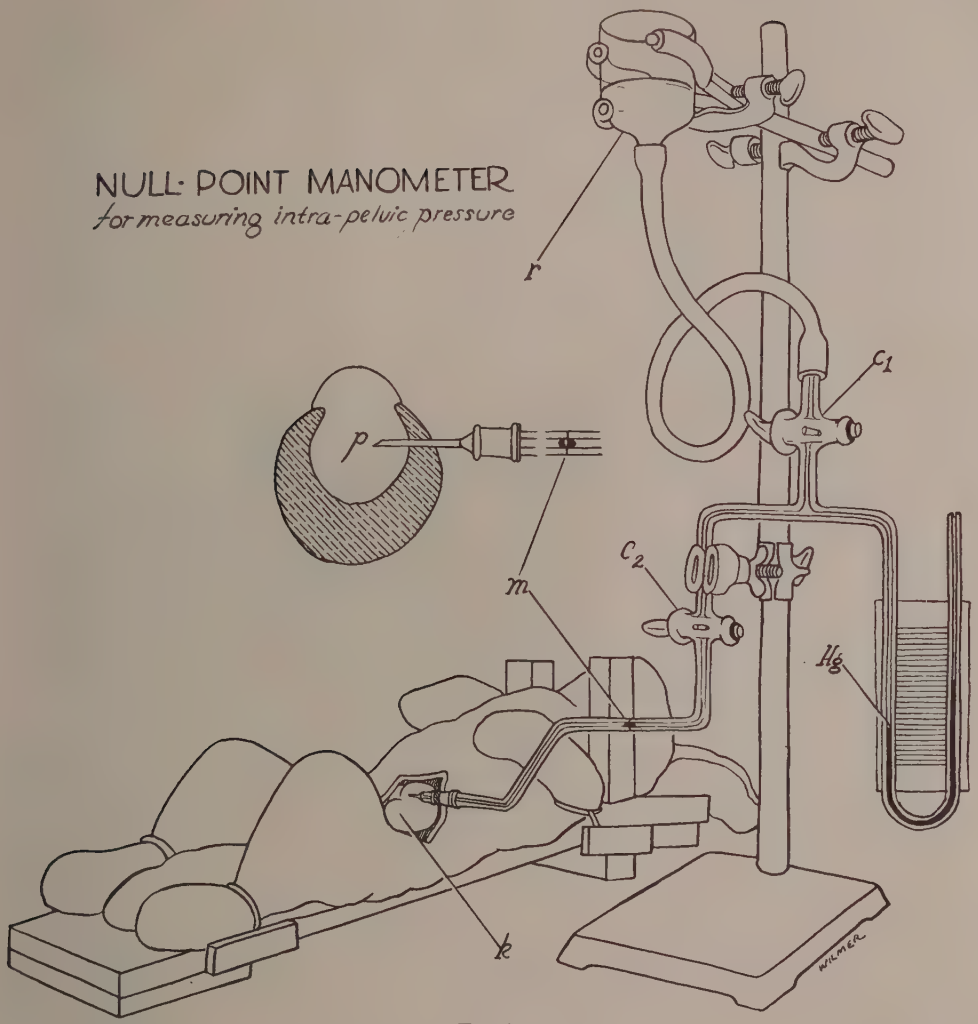

FIG. 1.

Null Point Manometer. This apparatus consists of a capillary tube bent into a deep "U" forming a mercury manometer in one arm, while the other arm is so bent that a 20-gauge needle adapted to it can be inserted into the renal pelvis as indicated in the insert. The entire capillary system is filled with normal saline with the exception of the mercury manometer and a single drop of mercury in the horizontal portion of the opposite arm. A reservoir above the system permits the pressure to be changed. Stop-cocks permit varying the pressure without sudden changes in the pelvis. When a pressure is found which exactly equals that in the hydronephrotic sac the mercury drop stands precisely still at the scratch mark on the glass where it stood at the beginning of the experiment.

of the distended fibromuscular pelvis. One estimates the pressure to be measured and then establishes this pressure in the manometer system. Repeated readings are made until the pressure in the system equals that in the pelvis. The accuracy of the method is due to the fact that the fluid content of the hydronephrotic sac is the same at the end of the experiment as at the start. The measuring system (Fig. 1) is employed in the sense of a balance.

The urine from the hydronephrotic sac and from the bladder was examined in each animal and contained no blood, and with 2 exceptions

TABLE I.
Static Intrapelvic Pressures and Specific Gravity.

Rabbit Exp. No.	Duration hydronephrosis days	Intrapelvic pressure, mm Hg.	Specific gravity	
			Right (unoperated)	Left (hydronephrotic)
R-1	3	7.5	qns	1.024
R-2	6	27.5	qns	qns
R-3	11	22.5	1.029	1.013
R-4	16	23.5	1.029	1.010
R-5	23	unsat.	1.018*	1.017*
R-6	28	17.5	1.020	1.007
R-7	37	20.0	1.029	1.008
R-8	63	16.0	1.021	1.002

* Unfiltered.

was perfectly clear. In one rabbit the fluid content of the sac was too viscid to interplay freely with the saline in the measuring system (Rabbit 5). The specific gravity of the urine was determined with the Gay-Lussac picnometer after passing through a sinter filter. Corrections were made for temperature, and an analytical balance used for determinations. The specific gravity readings show that the hydronephrotic kidney rapidly loses its ability to concentrate urine, and with the exception of a single reading of 1.002 the results are in agreement with previous work.

Discussion. The static intrapelvic pressure is quite different from the secretory pressure. While the static pressure is maintained physiologically *in vivo* the secretory pressure is the pressure of a column of urine or mercury against which the kidney can secrete. Surprisingly, this secretory pressure may reach 125 mm Hg.[5] The static intrapelvic pressure was found to be 7.5 mm Hg. at 3 days, to rise to between 22.5 and 27.5 mm Hg, in the interval of 6 to 16 days and to fall slightly thereafter to 16 mm Hg, at 63 days. (Table I). These pressures are below the effective glomerular filtration pressure, and therefore serve only to suppress and not stop urine formation. Why only small quantities of urine reach the pelvis following release of this back pressure[6] is not known. Under these circumstances the factors causing a pyelovenous reflux are no longer existent. Yet, it is many weeks before the urine volume becomes appreciable.[6] Histologically the glomeruli are normal, but there is tubular disturbance. This suggests that there might be considerable back diffusion through the convoluted tubules. Our experiments do not confirm or deny the pyelovenous reflux, but are compatible with it, if such refluxes occur at the pressures we have recorded. The function of the hydronephrotic kidney cannot be resolved into hydrostatic pressures without making assumptions based upon theories without conclusive experimental evidence.

Summary. The static intrapelvic pressure of the hydronephrotic kidney varies from 7.5 mm to 27.5 mm Hg. at various intervals from 3 to 63 days. Because of the technical accuracy of the Null Point Manometer employed in this study the readings reported here are considered exact for anesthetized rabbits at the moment of hydronephrosis at which they were determined.

[5] McDonald, J. R., Mann, F. C., and Priestly, J. T., *J. Urol.*, 1937, **37**, 326.

[6] Johnson, R. A., *J. Exp. Med.*, 1918, **28**, 193.

14591

Methemoglobin: A Normal Constituent of Blood.*

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The conversion of hemoglobin to methemoglobin by various chemical compounds and drugs has been reported repeatedly. Methemoglobin, so produced, has been held responsible for the toxic symptoms associated with its presence in the blood stream. However, there is little conclusive evidence that methemoglobin is the single causative factor in such toxemias. Methemoglobin may be a secondary factor in the chain of events associated with its presence in the blood stream.

The present work was undertaken to ascertain whether or not methemoglobin could be detected in the blood of normal persons, and to establish its normal range of concentration if it could be shown to be present. It was felt that either an affirmative or a negative answer to this question would have some bearing upon the problem of correctly interpreting the role of methemoglobin in pathologic conditions. If methemoglobin is a constituent of blood in normal persons, even in small concentrations, the view that it, in itself, is not toxic, and that its presence in higher concentrations in pathologic conditions is secondary to other factors which are responsible for the toxic symptoms, would gain support.

Experimental. Determinations were made upon two groups: (A) One hundred patients were selected at random from the wards of the Department of Medicine of the University of Iowa Hospital. No degree of selection was exercised other than to exclude those patients who were receiving sulfa drugs or other recognized methemoglobin-formers. The distribution in terms of sex was about 3 males to one female. The ages of the patients ranged from 7 to 78 years; no one age group predominated. A wide range of diseases was represented in order to rule out the possibility that methemoglobinemia was part of any given pathologic condition.

Blood samples were obtained by venipuncture from the forearm, using potassium oxalate as the anticoagulant. All blood samples were taken immediately to the laboratory, and, in most cases, complete analysis was made in 15 to 20 minutes. In no case did the period between withdrawal of the blood and completion of the analysis exceed one hour.

The method of analysis was that of Michel and Harris,[1] using a Coleman spectrophotometer, model 10S, and a Coleman electrometer, model 3E. This method utilizes the change in the extinction coefficient on conversion of methemoglobin to cyanmethemoglobin. One ml of whole blood is pipetted into a 25 ml volumetric flask. The blood is hemolyzed by the addition of a few milligrams of saponin. It is then diluted to approximately 23 ml with distilled water. One and one-fourth ml of 1M phosphate buffer, pH 7.4, is then added, and the solution is diluted to 25 ml. Without filtering, the extinction coefficient is ascertained on a portion of the sample of 634 mu. Two to 4 mg of dry KCN are then added to the solution in the spectrophotometer cuvette, and the contents mixed by inversion. One minute later the extinction coefficient is again ascertained, and the methemoglobin calculated as follows:

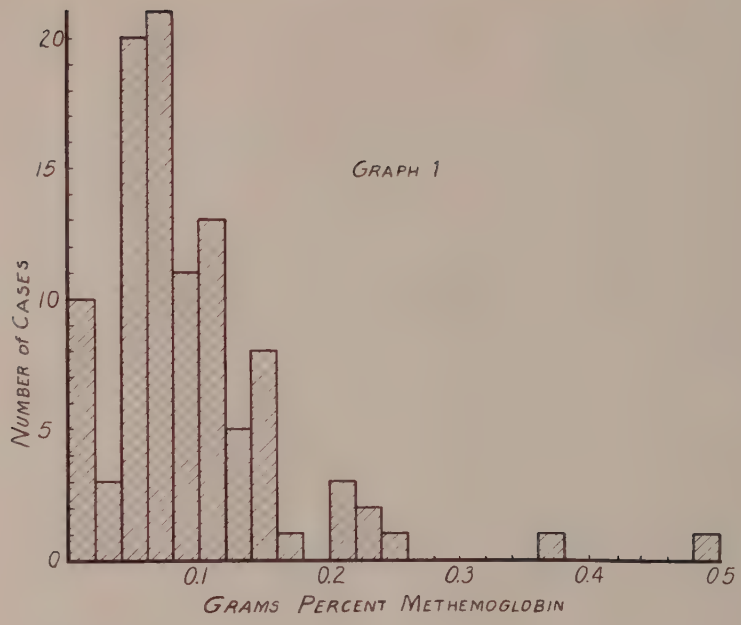
$$\text{Grams \% methemoglobin} = \frac{E634 - E634 \text{ with cyanide}}{F}$$

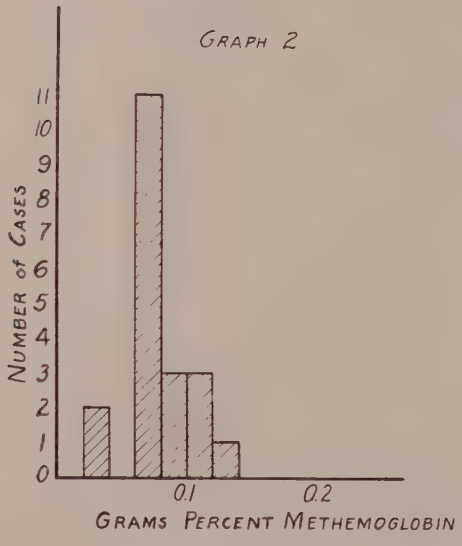
where $F = 0.0728$ at pH 7.4

(B) Twenty blood samples were obtained directly from donors to the blood bank of the University Hospitals. This group, therefore, was highly selected, in that no known pathologic condition existed, and no known medication preceded the estimation. All other factors in this series of estimations were

* Aided by a grant from the Institute for the Study of Sedative and Analgesic Drugs.

1 Michel, H. O., and Harris, J. S., *J. Lab. and Clin. Med.*, 1940, **25**, 445.





identical with those of group (A).

Results. Methemoglobin was found in the plasma of all patients except one. The values in the remaining cases ranged from 0.01 to 0.5 g %. Thirteen percent gave values ranging up to 0.04 g %, and 9 % of the group ranged from 0.16 to 0.38 g % of methemoglobin. The mean methemoglobin for one hundred cases was 0.09 g %. Graph I shows the distribution of methemoglobin levels for this group. No correlation was observed between methemoglobin and hemoglobin levels.

In group (B) methemoglobin was detected in the blood of all donors studied. The minimum amount was 0.03 g %; the maximum, 0.13 g %. Graph II shows the distribution of methemoglobin values in this group.

14592

Nitrogen Balances on Rats During Pregnancy and Lactation.

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The present investigation is a continuation of previous studies carried out in this laboratory on the balances of elements in rats during the period of pregnancy and lactation.[1] It has not yet been possible to maintain rats on synthetic diets for successive generations with production of normal litters.[2] This probably indicates that these diets are not adequate in all respects. It was hoped that, by comparing the nitrogen balances obtained when the animals were fed various synthetic diets with similar data when the rats were maintained on a stock ration that has proved adequate for the colony, some information as to the required dietary factors could be obtained.

Adult female rats weighing over 200 g and having a record of one or 2 litters when maintained on the stock diet were employed in the present experiments. The animals were placed on the experimental diets for a period of 3 weeks or until they had attained weight equilibrium before the balance experiments were initiated. Throughout the experiments food and water were supplied *ad lib*. The amount of food consumed was determined by weighing. The excreta were collected in 0.5 N H_2SO_4 to prevent loss of nitrogen. Balance experiments were carried out during a fore-period of 3-8 days before mating. These were resumed subsequent to mating. The litters were reduced to 6 in all cases. They were weaned at about 21 days. The amount of nitrogen loss to the mothers as a result of littering was determined by estimating the nitrogen content of one or more of the young shortly after birth. The amount of nitrogen supplied by the mother to the litter during lactation was estimated by determining the nitrogen content of one or more of the young at the time of weaning and subtracting from this value the nitrogen content of the young at birth. It is not possible to separate the excreta of the litter from that of the mother during the lactation period. Since the amount of nitrogen gained by the litter was taken to be L_{w-b}, no error results by including the excreta of the litter with that of the mother. The nitrogen balances were followed on the mothers for 7-14 days after removal of the young.

The following diets were fed: Group I, washed casein, 27 (increased to 30 for rats in the 300 series); cane sugar, 52; salt mixture,* 4; crisco, 7; corn oil, 7%. To this was added (mg per kilo) thiamin, riboflavin, and pyridoxin, 6 each; nicotinic acid, 30; pantothenic acid, 60; inositol, 200; 2-methyl-1,4-naphthoquinone, 0.05; choline chloride, 500; *p*-aminobenzoic acid, 5. In addition the diet contained 12 cc wheat germ oil, 5,000 μ vitamin D, and 65,000 μ vitamin A per kilo. The Group II rats received the same diet as the animals in Group I except that in addition each animal was given 50 mg *dl*-methionine daily. The Group III rats were maintained on the Group I diet to which 3% liver extract (Lilly)† had been added. The Group IV animals were maintained on the stock diet the composition of which was as follows: skimmed milk powder, 3.5; corn, 6; wheat germ, 2.25; whole wheat, 8; fish meal, 4.25; alfalfa meal, 1.25; lard, 1.5; NaCl and $CaCO_3$, each 0.125 lb; and fish liver oils, 50 cc. The average per cents of nitrogen in the diets were: diet I (27% casein) 3.3 and (30%

1 Goss, H., and Schmidt, C. L. A., *J. Biol. Chem.*, 1930, **86**, 417; Heppel, L. A., and Schmidt, C. L. A., *Univ. of Calif. Pub. Physiol.*, 1938, **8**, 189.

2 Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED. 1940, **45**, 625. In this connection see also Vinson, L. J., and Cerecedo, L. R., *Arch. Biochem.*, 1944, **3**, 389.

* The salt mixture contained: ferric citrate, 31.9; calcium lactate, 352; $Ca(H_2PO_4)_2 \cdot H_2O$, 146; K_2HPO_4, 258; $NaH_2PO_4 \cdot H_2O$, 93.8; NaCl, 46.7; $MgSO_4 \cdot 7H_2O$, 156; $MnSO_4 \cdot 4H_2O$, 1.3; NaI, 2.0; $Ca_3(PO_4)_2$, 132; $CuCO_3$, 2.0 g.

† Kindly supplied by Eli Lilly and Company.

TABLE I.

Foreperiod			Period of pregnancy						Period of lactation						Afterperiod			
Rat No.	Days	Bal.	Days	I	L_b	O	$O + L_b$	Bal.	Days	I	L_{w-b}	O	$O + L_{w-b}$	Bal.	Days	Bal.	Days	Bal.
Group I (Synthetic diet)			Totals for period								Totals for period							
11	3	+0.06	21	11.42	0.63	10.51	11.14	+0.28			Failed to lactate							
3	3	—0.03	21	11.71	0.70	11.58	12.28	—0.57			,, ,, ,,							
6	3	+0.06	21	7.20	0.38	8.42	8.80	—1.60			,, ,, ,,							
103	3	+0.02	21	8.62	0.22	9.68	9.90	—1.28	21	9.77	3.57	9.04	12.61	—2.84				
104	3	+0.10	21	8.73	0.61	9.14	9.75	—1.02	21	10.99	2.31	11.29	13.60	—2.61				
305	8	—0.08	22	12.19	0.64	11.97	12.61	—0.42	20	16.27	4.34	12.95	17.29	—1.02	9	—0.41		
311	7	—0.04	22	12.36	0.68	12.51	13.19	—0.83	21	18.33	4.39	12.30	16.69	+1.64	7	—0.07		
Group II (Synthetic diet + methionine)			Totals for period								Totals for period							
319	7	—0.02	22	12.63	1.32	11.59	12.91	—0.28	21	16.23	2.97	15.90	18.87	—2.64	8	+0.78	7	+0.33
320	7	—0.01	22	9.47	1.04	9.42	10.46	—0.99	21	15.04	2.36	13.73	16.09	—1.05	7	+0.19	7	—0.11
321	7	+0.10	22	10.15	0.77	9.46	10.23	—0.08	21	15.73	3.60	13.26	16.86	—1.13	8	+0.55	7	—0.20
325	7	+0.02	22	11.29	0.70	10.71	11.41	—0.12			Failed to lactate				7	+0.18		
Group III (Synthetic diet + liver extr.)			Totals for period								Totals for period							
18	3	+0.12	21	11.73	0.85	9.77	10.62	+1.11	22	21.48	5.34	13.00	18.34	+3.14				
10	3	+0.18	21	13.93	0.81	12.18	12.99	+0.94	23	22.30	6.02	16.62	22.64	—0.34				
102	3	+0.12	21	12.20	0.65	9.78	10.43	+1.77	22	17.48	5.72	13.38	19.10	— 1.62				
312	7	—0.17	22	11.31	0.84	8.84	9.68	+1.63	21	17.23	1.72	19.51	21.23	—4.00	7	—0.07		
323	7	0	22	12.04	1.30	9.18	10.48	+1.56	20	15.81	4.38	11.28	15.66	+0.15	9	+2.76	8	—0.15
324	7	+0.10	23	11.44	1.61	8.86	10.47	+0.97	20	18.95	5.00	13.08	18.08	+0.87	8	+0.52	7	+0.09
Group IV (Stock diet)			Totals for period								Totals for period							
105	3	+0.07	21	11.76	0.62	10.44	11.06	+0.70	21	21.06	5.25	16.83	22.08	—1.02				
106	3	+0.06	21	14.36	0.84	12.42	13.26	+1.10	22	29.04	6.62	18.69	25.31	+3.73				
107	3	—0.01	21	11.69	0.81	9.18	9.99	+1.70	23	21.82	5.75	17.94	23.69	— 1.87				
310	7	—0.07	22	12.32	0.67	11.52	12.19	+0.13	21	19.41	4.21	11.80	16.01	+3.40	7	—0.84		
326	7	—0.09	21	10.63	0.73	8.08	8.81	+1.82	21	19.75	4.37	14.23	18.60	+1.15	6	+0.05	7	—0.12
327	7	—0.16	22	11.43	0.78	8.57	9.35	+2.08	21	14.77	3.25	12.64	15.89	—1.12	7	+0.25	7	+0.14

I = input of nitrogen; O = output of nitrogen; L_b = nitrogen content of litter at birth; L_{w-b} = nitrogen content of litter at weaning minus that at birth; O + L = total nitrogen output; all nitrogen values are in g.

casein) 3.9; diet III, 3.8; diet IV, 3.3.

The data are summarized in Table I. Although the input and output of nitrogen were determined during weekly periods the values are not included in the table since they are quite variable and show no particular trends. The data are, however, on file.

Analysis of the data shows the following: (a) All of the Group III and Group IV rats had gained nitrogen at the end of the period of pregnancy while with one exception those of Groups I and II had lost nitrogen. During this period all animals gained weight as would be expected. (b) Most but not all of the animals in Groups III and IV gained nitrogen at the end of the lactation period while with one exception the Groups I and II rats lost nitrogen. Three animals (103, 305, and 311) in Group I, and 2 (319 and 321) in Group II lost weight during the lactation period while one in each of these 2 groups maintained its body weight. Of the Group III rats, 4 maintained their body weights, 1 (323) lost and 1 (312) gained weight. Five animals of Group IV lost weight and 1 (105) maintained its weight. There was no correlation between loss or gain in body weight and loss or retention of nitrogen during lactation in any of the groups. (c) Examination of the weekly nitrogen balances of the Group III and Group IV rats shows no uniform correlation between nitrogen retention and development of the fetus although in many cases the maximum nitrogen retention occurred in the third week of pregnancy. In Groups I and II there is no correlation between loss or retention of nitrogen and the period of pregnancy. Similar statements may be made of the lactation periods of all groups of animals. (d) Under the conditions of the experiment and of the diets studied the liver extract supplemented diet compares most favorably with the stock ration in preventing loss of nitrogen in pregnant and lactating rats. This may be indicative of the presence of one or more factors in liver extract required by rats during pregnancy and lactation; however, direct proof is lacking

Although a great many studies on nitrogen metabolism during pregnancy and lactation have been published, apparently none of them have dealt extensively with the rat.‡ For women the experiments of Macy and her coworkers[3] are representative. They found that the reproductive cycle is a period of nitrogen acquisition for the mother although at the time of delivery and in the early period of lactation the nitrogen balance may be negative. On the other hand Murlin[4] found in dogs that nitrogen loss occurs during the first half of pregnancy and particularly in the third and fourth week while nitrogen is retained in the last half of pregnancy. The adequacy of the diet used by Murlin may be questioned. Campbell[5] in this laboratory showed that rats, maintained on a diet containing 2.4% nitrogen but less adequate in the vitamins than in the present experiments, stored amounts of nitrogen that met the needs of the fetus. When maintained on a similar diet but containing only 1.2% nitrogen the amount of nitrogen retained was not adequate for the requirements of the fetus. During the lactation period the animals on the first mentioned diet were in negative nitrogen balance but were able to rear their young while those maintained on the diet of lower nitrogen content were unable to do so unless at the onset of the lactation period there was an unusually large reserve of nitrogen upon which to draw.

Summary. Nitrogen balances have been carried out on rats maintained on various diets during the period of pregnancy and lactation. The liver extract supplemented diet compared most favorably with the stock ration in preventing loss of nitrogen.

‡ In this connection see Poo, L. J., Lew, W., and Addis, T., *J. Biol. Chem.*, 1939, **128**, 69.

[3] Hunscher, H. A., Donelson, E., Nims, B., Kenyon, F., and Macy, I. G., *J. Biol. Chem.*, 1932-33, **99**, 507; Hunscher, H. A., Hummell, F. C., Erickson, B. N., and Macy, I. G., *J. Nutr.*, 1935, **10**, 579.

[4] Murlin, J. R., *Am. J. Physiol.*, 1910, **27**, 177.

[5] Campbell, W. W., *The Nitrogen Balance in the Rat During Pregnancy and Lactation*, Thesis, Univ. of Calif., 1938.

14593

Influence of Hypothermia and Hyperthermia on Survival Time of Dogs in Hemorrhagic Shock.*†

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During the past 3 years researches in this laboratory[1] have convinced us (1) that hemorrhage which leads to severe and protracted hypotension leads to an irreversible state from which the animal dies after reinfusion of all the blood withdrawn, (2) that the cardio- and hemo-dynamic course of events is identical with that observed in other types of shock, and (3) that shock so produced by standardized bleeding eventuates in death within 2-8 hours in 75 to 80% of dogs under morphine-barbital anesthesia or, expressed otherwise, may be said to offer an animal a 20-25% chance of maintaining their arterial pressures at reasonably normal levels for 4 to 6 hours.

This report concerns itself with the use of the method for comparing the effects of hypo- and hyperthermia on total survival time. The method for producing standardized hemorrhagic shock in dogs anesthetized with morphine and barbital has been described previously.[1] It consists, briefly, in withdrawing blood rapidly until mean arterial pressure has fallen to 50 mm Hg., in holding it at this level for 90 minutes by repeated small withdrawals of blood, in subsequently reducing mean arterial pressure to 30 mm Hg. for 45 minutes, in reinfusing the withdrawn blood (heparinized)‡ and observing the trend of arterial pressures 4-6 hours thereafter. If the pressure declined to 50 mm Hg. or the animal died, shock had occurred; if the animal maintained normal blood pressures 6 hours or better, a nonshock state was declared to exist. In the present experiments *the time of survival* after reinfusion of blood was chosen as a criterion. Operative procedures were kept at a minimum and consisted only in cannulation of the femoral arteries for bleeding and registration of mean arterial pressures and of a femoral vein for reinfusion.

Survival times of hyperthermic animals submitted to standardized bleeding were available in the case of 17 animals previously used by other groups in this laboratory[1] during the hot summer months of 1942 when room temperatures ranged from 26 to 33°C and rectal temperatures of the dogs varied from 38.8 to 40.1°C. To these were added 7 more animals studied under similar conditions during hot summer days in 1943. Their rectal temperatures ranged from 38.3 to 41.7°C.

For comparison, a series of 20 dogs was submitted to the standardized bleeding while their bodies were cooled in a semi-insulated box at environmental air temperatures of 15-20°C, and with radiating wall surfaces slightly above that of melting ice. In a few instances, ice bags were also applied for short intervals of time when it seemed desirable to hasten the reduction of body temperature.

In all of these experiments the temperature of the box air, of the trunk, extremities, ears, and rectum were continuously recorded at 6-minute intervals by a "Micromax" recorder. It was found difficult, however, to maintain animals in such cooled environments at a strictly constant temperature owing to changes in arterial pressure, blood flow in the skin and

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† Condensed summary of results presented as a thesis for partial fulfillment of requirements for the degree of M.S. in the Graduate School of Western Reserve University.

1 Werle, J. M., Cosby, R. S., and Wiggers, C. J., *Am. J. Physiol.*, 1942, **136**, 401; Wiggers, C. J., and Werle, J. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 604; Huizenga, K. A., Brofman, B. L., and Wiggers, C. J., *Idem*, 1943, **52**, 77; *J. Pharm. and Exp. Therap.*, 1943, **78**, 139; Wiggers, H. C., and Middleton, S., *Am. J. Physiol.*, 1944, **140**, 677.

‡ We are indebted to Roche-Organon, Inc., Nutley, N.J., for a generous supply of Liquaemin used in these experiments.

TABLE I.

Hyperthermic Animals. Dog No.	Effective mean temp., °C	Survival period, hr	Hypothermic Animals Dog No.	Effective mean temp., °C	Survival period, hr
17A	38.8	3.1	9C	28.3	2.5
10A	38.8	3.3	18C	29.4	24.8
15A	38.8	3.7	2C	30.6	13.3
3A	38.8	4.3	14C	30.4	*
5A	38.9	4.2	16C	30.6	13.0
12A	38.9	3.0	19.C	30.8	20.0
6A	39.0	7.8	3C	31.8	14.5
11A	39.2	5.5	5C	31.5	11.5
AA	39.2	12.0	7C	31.5	4.3
13A	39.2	13.0	17C	32.4	12.0
2A	40.0	2.5	4C	32.9	14.5
4A	40.0	2.8	1C	33.0	9.2
7A	40.0	4.9	20C	33.4	20.0
8A	40.0	5.4	15C	33.1	9.0
			13C	35.6	33.5
	40.1	2.8	11C	35.4	20.0
14A	40.1	4.5	5C	35.5	4.5
16A	40.1	4.8	12C	35.6	33.5
			8C	36.1	3.0
4B	38.3	12.0	10C	36.7	31.5
6B	38.5	4.8			
5B	38.9	3.5		Avg	15.4
2B	39.7	2.0		Range	3-33.5
7B	40.0	3.2		(excluding 14C)	
1B	40.3	11.5		Mode	13.9
3B	41.7	3.2		S	10.45
			*Sacrificed 6 days		
Avg		5.3			
Range		2-13 hrs			
Mode		4.25			
S		3.34			

metabolic rate produced by withdrawal and reinfusion of blood. Consequently, an "effective average rectal temperature" was calculated for each hypothermic animal by taking the average of the temperatures which obtained during the periods of 50 mm Hg. hypotension, of 30 mm Hg. hypotension and either of the 4 hours after reinfusion or of a lesser time when the animal died sooner.

Results. The results of all experiments on hyperthermic and hypothermic animals, all submitted to identical standardized bleeding, are summarized in Table I. These are rearranged in accordance with the effective average rectal temperatures of the animals during the experimental periods. A glance indicates that the average and most of the individual survival periods are definitely longer in animals whose rectal temperature was kept below 37°C than in those in whom it ranged above this level. The differences are statistically significant. On the other hand, it must also be noted that with one exception (Dog 14-C), all animals succumbed. Autopsies were performed on all but one of the cooled dogs, *viz.*, the one that lived for 6 days after the experiment. In all but 3 animals the duodenum showed mucosal changes varying from a slight streaky hyperemia (1+) to intense congestion, edema, and bloody fluid in the lumen (3+). In 13 animals this extended far down into the jejunum and ileum (4+). In 15 animals, intense congestion of the rectum and sigmoid was also present. In all except 6 of the animals subendocardial hemorrhages were found varying from a few small petechiæ, 1-3 mm in diameter, to large confluent hemorrhages, 1-3 cm in diameter. Hence, while cooling of the body tends to prolong life after damage due to prolonged severe hemorrhagic hypotension it does not lead to recovery under these conditions.

The practical value of such an extension of the survival time is debatable. It has often

been stated that it is the first duty of a physician to keep a patient alive so that he may be given a chance of being cured later. However, until such chances are increased beyond what they appear to be at present, in the case of our standardized hemorrhagic shock, the practical value of maintaining a somewhat lower body temperature appears vanishingly small. However, it must be kept in mind that the shock induced in our animals is of a severe form and the possibility exists that hypothermia may be of greater benefit in other types of shock.

Summary. Observations on 44 dogs submitted to standardized bleeding and reinfusion procedures which induce irreversible hemorrhagic shock showed that the survival period following reinfusion of heparinized blood is definitely extended by a hypothermic state, but with one exception all dogs succumbed.

2 Blalock, A., and Mason, M. F., *Arch. Surg.*, 1941, **42**, 1054; Elman, R., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 350; Rosenthal, S. M., *Pub. Health Rep.*, 1942, **57**, 1923; Wakim, K. G., and Gatch, W. D., *J. Am. Med. Assn.*, 1943, **121**, 903; Allen, F. M., *Am. J. Surg.*, 1943, **60**, 335.

14594

Metabolic Studies in Patients with Cancer of the Gastro-Intestinal Tract. XX. Lipotropic Properties of Protein.*

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Recent studies from these laboratories have demonstrated the value of protein-rich diets in the treatment of the hepatic dysfunction of patients with gastro-intestinal cancer. This conclusion has been based chiefly upon the increased functional capacity of the liver as determined by multiple function tests. The livers of these patients, when untreated, almost uniformly contain at laparotomy abnormally high concentrations of lipid and probably low concentrations of glycogen,[1] and there is reason to believe that this unusual chemical constitution, in turn, may account for a part of the reduced functional state of the organ.[2,3] In an earlier investigation it was demonstrated that the livers of patients with gastro-intestinal cancer readily could be depleted of their excess fat. One method by which this could be accomplished was by the oral administration of inositol or lipocaic merely during the night preceding operation.[4] For these reasons, it appeared desirable to learn if the return towards normal hepatic function induced by the feeding of high protein diets to the patients studied was associated with the restoration of a normal chemical composition of their livers.

Methods. The methods used in the present study have been described in previous communications.[1,4]

Results. In a previous investigation[1] the concentrations of lipid in the livers of 28 patients with gastro-intestinal cancer who came to laparotomy after the usual 10-hour preoperative fast ranged from 5.3 to 35.0 g and averaged 16.40 g %. The glycogen content of these livers, in turn, varied from 0.5 to 8.3 g and averaged 2.56 g %.

In contrast, the concentrations of fat in the livers of 7 patients with gastro-intestinal cancer who ingested 2.5 g of complete protein per kg per day for from 10 to 21 days, were all

* The authors gratefully acknowledge the assistance of a grant from the National Cancer Institute.

1 Ariel, I. M., Abels, J. C., Murphy, H. T., Pack, G. T., and Rhoads, C. P., *Ann. Int. Med.*

2 Conner, C. L., *Am. J. Path.*, 1938, **14**, 347.

3 Soskin, S., and Hyman, M., *Arch. Int. Med.*, 1939, **64**, 1265.

4 Abels, J. C., Ariel, I. M., Murphy, H. T., Pack, G. T., and Rhoads, C. P., *Ann. Int. Med.*

TABLE I.
Effects of Ingested Protein and Amino Acids on Chemical Composition of Livers of Patients with Gastro-Intestinal Cancer.

Dietary supplement	No. of patients	Hepatic conc. of lipid		Hepatic conc. of glycogen	
		Range, g %	Avg, g %	Range, g %	Avg, g %
None	28	5.3 -35.0	16.40	0.5-8.3	2.56
High protein (2.5 g/kg/day)	7	4.6 - 7.55	5.59	0.8-3.8	2.23
Amino acids (75 g)	9	9.05-26.2	15.45	1.3-3.3	2.39

within normal limits.[5] These values ranged from 4.6 to 7.55 g and averaged 5.59 g %, or 0.34 that of the fasted patients. The concentrations of glycogen in these livers, however, varied from 0.8 to 3.8 g and the average value was only 2.23 g % or even less than that of the fasted control group.

On the other hand, the chemical composition of the livers of 9 patients with gastro-intestinal cancer who received 75 g of amino acids† during the 10-hour period before operation were not significantly different from those of fasted patients. This amount of amino acids was chosen because it was the largest that could be given orally in a 10-hour period without inducing untoward reactions. The livers of this group were found to contain from 9.05 to 26.2 g % of lipid (average 15.45 g %) and from 1.3 to 3.3 g % of glycogen (average 2.39 g %).

Comment. It has become widely recognized that the administration of protein-rich diets to patients with hepatic insufficiency frequently results in considerable clinical benefit.[6] This, likewise, appears to be true in patients with gastro-intestinal cancer who have fatty infiltration of the liver. There is some reason to believe that the hepatic dysfunction of this group of patients may be due to the very presence of the excess fat of the liver cells. Lipid is deposited apparently without water[7] (in contrast to the deposition of glycogen[8]) so that the available hepatic water necessarily becomes distributed through a greater tissue volume, and the solution of essential water-soluble constituents thus is decreased. Moreover, an increased hepatic content of lipid probably favors the solution and retention of many lipid-soluble substances deleterious to the liver,[9] compresses the sinusoids, and induces ischemia.[2] It is possible, therefore, that it is the lipotropic effects of the protein which may account in part for the functional improvement of the organ.

Conclusions. 1. Patients with gastro-intestinal cancer fed protein-rich diets preoperatively do not have fatty infiltration of the liver at laparotomy. 2. Patients with gastro-intestinal cancer who ingested 75 g of amino acid mixture during the 10 hours before operation were found at laparotomy to have concentrations of fat as abnormally high as those of fasted patients.

5 Ralli, E. P., Rubin, S. H., and Rinzler, S., *J. Clin. Invest.*, 1941, **20**, 93.

† Acid hydrolysate of casein procured through the courtesy of Frederick Stearns & Co., Detroit, Mich.

6 Patek, A. J., Jr., and Post, J., *J. Clin. Invest.*, 1941, **20**, 481.

7 McLachlan, P. L., Hodge, H. C., Bloor, W. R., Welch, E. A., Truax, F. L., and Taylor, J. D., *J. Biol. Chem.*, 1942, **143**, 473.

8 McBridge, J. J., Guest, M. M., and Scott, E. L., *J. Biol. Chem.*, 1941, **139**, 943.

9 Goldschmidt, S., Vass, H. M., and Ravidin, I. S., *J. Clin. Invest.*, 1939, **18**, 277.

14595

A Study of Anuria in Experimental Shock.*

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In experiments on the effect of thermal trauma on the G.I. tract it was frequently observed that the urine output after burns was low even after large infusions of saline. While this has been known some time,[1] no comparative study has been made on anuria in different types of shock, using approximately the same experimental approach in each.

Experimental Procedure. Large dogs were used for this study and all animals were deeply anesthetized throughout the experimental period with nembutal or sodium barbital. An excess of anesthetic was administered at the termination of each experiment. Each ureter was cannulated and connected to a sensitive drop recorder which registered on a kymograph the drops of urine from each kidney.[2] Each drop of urine represents 0.052 cc. Blood pressure was recorded on the same kymograph so that an immediate correlation between the effects of shock on blood pressure and urine secretion was possible. Repeated determinations of plasma CO_2, hemoglobin, hematocrit, and, in some experiments of serum N.P.N. were made. Hemorrhagic, burn, and traumatic shock were studied. Duncan-Blalock clamps[3] were used for the production of traumatic shock because this method produces little if any hemorrhage.

Results. The total of 43 experiments may be divided into 4 groups. (1) Two control experiments of 18 and 32 hours duration. (2) Five hemorrhage experiments. (3) Twenty-three thermal trauma experiments. (4) Thirteen traumatic shock experiments. Analysis of the experiments is done most effectively by examining and comparing individual experiments of each group. Therefore, from the 43 experiments 8 will be discussed in detail because they illustrate the most consistent findings. However, the remaining 35 experiments deviated slightly from them by the severity of the anuria and oliguria. The extent of kidney damage was frequently dependent on the degree of injury (thermal, traumatic, or hemorrhagic) suffered by the animal, and the duration of time before treatment of the shock. But it was apparent that in most experiments the type of anuria or oliguria could be divided into definite characteristic groups.

Several interesting observations were made in the 2 control experiments. In the first control, without infusion, the kidneys secreted well for 17 hours. At that time the following changes occurred: blood pressure had dropped only 10 mm Hg. (to 115), hematocrit increased 11% (to 64%), CO_2 had fallen 8 vol. % (to 39 vol. %), and a decrease of urine secretion to 22 drops per 15 minutes from the control average of 30 drops. In the second control experiment, without infusion, the following changes occurred in the animal after a 21-hour period: Blood pressure dropped to 95 mm Hg., hematocrit had risen to 64%, the CO_2 had fallen to 18 vol. % from the control value of 50 vol. %, and the urine decreased to 16 drops per 15 minutes from the control value of 28 drops per 15 minutes. Five hundred cc of plasma i.v. increased urine secretion to 95 drops. These experiments demonstrated that the operative procedure, anesthesia, and lack of asepsis did not produce anuria in 17 or in 21 hours. The kidneys responded immediately to an infusion of plasma after this time. The second experiment was continued for an additional 11

* This work was done under the auspices of the Committee on Research in Shock, of Michael Reese Hospital, and was supported by The Michael Reese Research Foundation and by the Isaac and Kate Meyer Fund.

1 Harkins, Henry N., *The Treatment of Burns*, Charles C. Thomas, 1942, 33.

2 Olson, Wm. H., and Necheles, H., *J. Lab. and Clin. Med.*, 1942, **27**, 802.

3 Duncan, G. W., and Blalock, Alfred, *Surg.*, 1942, **115**, 684.

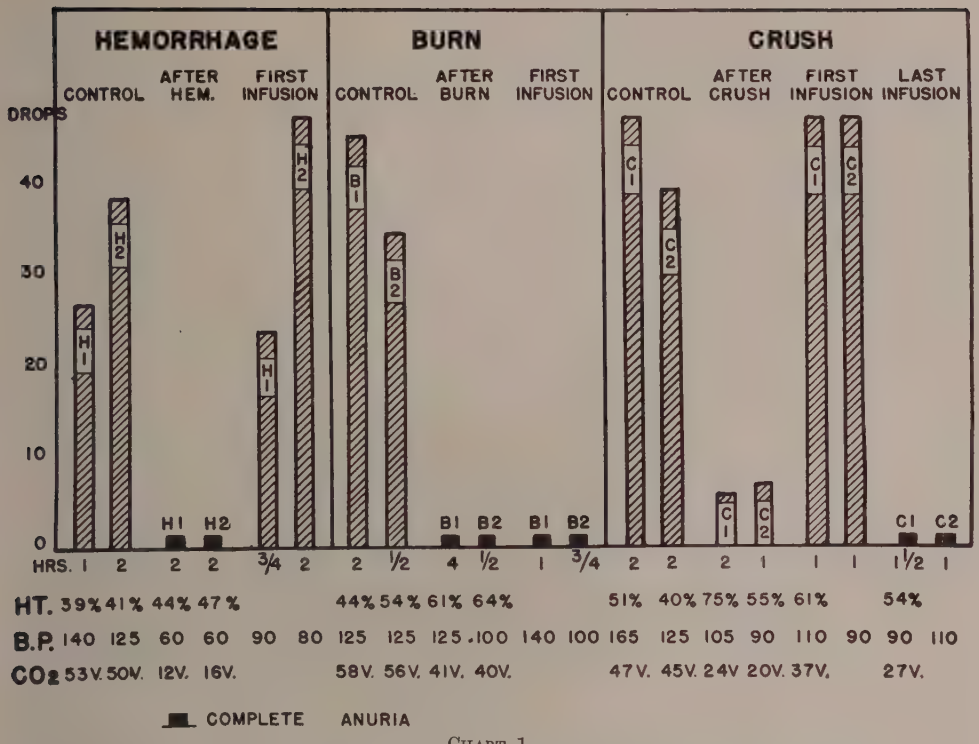

CHART 1.

Data of 6 experiments representing 3 types of anuria. The columns represent average urine secretion of both kidneys in drops per 15 minutes during the interval of time (Hrs) marked below each column. The values below each column are hematocrit, blood pressure in mm Hg and arterial plasma CO_2 in volumes percent. Control values were determined before and values during shock after the time interval. C1 and C2 secreted a total of 210 and 130 drops of urine respectively after infusion (not drawn fully on chart).

hours, when the animal died. The kidneys secreted following all subsequent infusions of plasma. No complete anuria was observed until shortly before death.

Chart 1 gives the significant data for 6 experiments, two experiments from each of the 3 types of shock studied. The first hemorrhage experiment (HI) was performed under nembutal on a dog weighing 20 kg. Control BP 140, hematocrit 39, and CO_2 53. During one hour, the average urine secretion was 27 drops per 15 minutes. The animal was bled 700 cc and the blood pressure dropped to 90. During the next 1½ hours, the average secretion was 26 drops per 15 minutes and the BP rose to 100. This period is not shown on the chart. The animal was then bled 375 cc and urinary secretion was completely suppressed for 2 hours. At that time blood pressure was 60, hematocrit was 44, and CO_2 12. A saline infusion of 300 cc raised BP to 90 and for ¾ hours stimulated a urinary secretion of 23 drops per 15 minutes; then the animal died. The second hemorrhage experiment (H2) was performed on an 18-kg dog under nembutal. Control BP was 125, hematocrit 41, and CO_2 50. For 2 hours the control urine secretion was 38 drops per 15 minutes. Then, the animal was bled 450 cc; BP dropped to 50, and for ¾ hours the average urine secretion was 10 drops per 15 minutes. During this time the BP rose to 85. The animal was then bled an additional 270 cc. BP dropped to 30, and urine secretion was completely suppressed for 2 hours. A 270 cc saline infusion, however, stimulated an average urine secretion of 50

drops per 15 minutes for 2 hours. BP rose to 80 mm Hg. The animal was then sacrificed.

Burn experiment B1 was performed on a 19.5 kg dog under nembutal. Control BP was 125, Ht. 44, CO_2 58, and urine secretion was 47 drops per 15 minutes. Thermal trauma by a torch was applied for 10 minutes. The BP rose to 155 during this time, and urine secretion stopped completely. Four hours later the Ht. was 61, BP 125, and CO_2 41. The plasma contained 1385 mg % of Hb. No urine was secreted during this time. A 300 cc saline infusion had no effect on the anuria, although the BP rose to 140. However, a second infusion, not shown in Chart 1, produced a urine secretion of 10 drops per 15 minutes for 1 hour. At this time the animal was sacrificed.

Burn experiment B2 was performed on a 15.5 kg dog under morphine-sodium barbiturate anesthesia. Control Ht. was 54, BP 125, and CO_2 56. During ½ hour control period, the average urine secretion was 35 drops per 15 minutes. Then a severe thermal trauma was administered. Urine secretion was completely inhibited. At ¾ hours after the burn the Ht. was 64, BP 100, CO_2 40, and urine secretion 0. The plasma contained 3680 mg % Hb. The animal was given an infusion of saline, 6 cc per minute. After 270 cc had been given, the rate of infusion was increased to 12 cc per minute. Blood pressure was 100. An additional 2160 cc of saline was given over a 3-hour period. No urine was secreted during this period, which terminated with the animal's death.

Crush experiment C1 was done on an 18 kg dog under nembutal. Control Ht. was 51, BP 165, CO_2 47, and average urine secretion over a 2-hour period was 48 drops per 15 minutes. Two Blalock clamps were applied for 5 hours, during which time the average urine secretion was 31 drops per 15 minutes (not shown on this chart); for a 2-hour period after removal of the clamps it fell to 6 drops per 15 minutes. After that period, the Ht. was 75, BP 105, and CO_2 24. The first infusion of 500 cc of plasma produced an average urine secretion of 210 drops per 15 minutes for a period of one hour, and of 30 drops per 15 minutes during the next 3 hours (the latter not shown on the chart). The response of the kidney to plasma infusion decreased slowly. At 19 hours after the first infusion, Ht. was 54, BP 90, and CO_2 27. However, even after a 500 cc plasma infusion, no urine was secreted. Complete anuria was present. This animal was sacrificed 1½ hours later, when its BP was 100.

Crush experiment C2 was done on a 15 kg dog under nembutal anesthesia. Control values were: Ht. 40, BP 125, CO_2 45, and average urine secretion for 2 hours was 40 drops per 15 minutes. Two clamps were applied for 5 hours. For one hour after the clamps were removed the average urine secretion was 7 drops. After that period the Ht. was 55, BP 90, and CO_2 20. The first infusion of 500 cc of plasma stimulated an average urine secretion of 130 drops per 15 minutes for one hour. At each subsequent plasma infusion, the kidneys secreted less, so that at 17 hours after the first infusion no urine was secreted after a 500 cc plasma infusion. The blood pressure rose to 100 during the infusion, but the animal died one hour later.

Discussion. In the present series of experiments we have attempted to find the conditions under which complete anuria would occur, and therefore kidney function was determined only by the volume of urine secreted.

The immediate effects of the 3 different types of shock on urine secretion was a marked depression. In the crush experiments the injury did not immediately produce complete anuria. Undoubtedly, if the BP had dropped lower, as in the hemorrhage experiments, an early anuria would have been present.

By the reactions to intravenous infusions, 3 different types of anuria became evident. The first type is produced mainly by low blood pressure and a reduced blood volume, such as occurred after hemorrhage and immediately after crushing. Infusion stimulated urine secretion in all these cases, regardless of the severity of the shock. In the hemorrhage experiments the BP was 60 and the CO_2 at a very low level: in one experiment the animal was infused shortly before death; yet, in all cases the infusion stimulated urinary secretion. In the early stages of the crush experi-

ments, urinary secretion was similar to that following hemorrhage. During this stage, considerable hemoconcentration (75% in one of the experiments) was present, yet the infusion stimulated considerable kidney secretion. It seems evident from these 2 groups of experiments that acidosis and hemoconcentration are not the important factors in complete anuria of shock. We may therefore assume, that reduced blood volume, hemoconcentration, low blood pressure, and acidosis do not lead to a permanent anuria, *i.e.*, one which is not abolished by infusions.

The second type of anuria may be called burn anuria. This type did not always respond to an infusion fluid. In experiment B1 the kidneys secreted urine only after the second infusion. In experiment B2 a total amount of 2,430 cc of saline had no effect on kidney secretion. The difference in response might be attributed to different amounts of hemoglobin in the plasma. In B1 the plasma hemoglobin was 1385 mg %, whereas in experiment B2 it was 3680 mg %. The severity of the anuria in all burn experiments frequently could be associated with the degree of hemoglobinemia.

The third type of anuria may be called crush anuria. It should not be mistaken for the first type, *i.e.*, the anuria of low blood pressure and diminished blood volume. In crush anuria, the kidneys always secreted well after the first infusion as in the first type, but the urine was deeply pigmented, presumably with myohemoglobin.[4] This was a sign of impending damage to the kidneys. Unlike following burns, the anuria developed very slowly and only after 18 hours or longer the kidneys might stop secreting, even after infusion. It was important to give infusions in order to determine the extent of damage to the kidneys. It seemed possible to prevent the crush anuria by giving certain infusion fluids early. Saline and plasma did not prevent it in all cases. In many cases complete anuria did not occur either in burns or in crush injuries, but all experiments exhibited some degree of oliguria.

Summary and Conclusions. I. Three different types of anuria were observed in these experiments.

1. Anuria following a severe hemorrhage or a reduced blood volume.

2. Anuria following a severe burn.

3. Anuria following a crush injury: this type developed much later, 18 to 36 hours, after injury.

II. The possible factors for the appearance of anuria are: low blood pressure, a reduced blood volume, the hemoglobinemia present in burns, and an unknown substance. This substance may be myohemoglobin or a toxic agent released from the damaged limb.

III. Permanent damage to the kidneys and anuria may develop following crushing or burning, because renal function seems to be most impaired following these two traumatic agents.

[4] Morison, J. Edgar, *Brit. Med. J.*, 1942, **2**, 736.

14596

Absorption of Galactose by Renal Tubules of the Dog.

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In previous papers from this laboratory evidence was presented to show that in the rat and in man, thyroid hormone increases the rate of the intestinal absorption of substances that are transferred actively by a mechanism generally considered to involve phosphorylation; the hormone was without influence on the rate of absorption of those substances that

are considered not to undergo phosphorylation.[1,2] Our[3] interest in the manner by which thyroid hormone exerts an influence on the mechanism involved in the active transfer of solutes across membranous barriers has led to a study of the effect of this hormone on renal tubular transfers. In a recent publication[4] we reported that thyroid hormone accelerates the active transfer (absorption) of glucose by the renal tubules of the dog. The observation that thyroxin also accelerates the active tubular transfer of diodrast, together with other considerations, led us to postulate that thyroid hormone may exert its characteristic effect on metabolism and on transfer mechanisms by increasing the effective concentration of adenosinetriphosphatase. Additional data supporting this hypothesis is now available.[5] In this report the comparison of intestinal and renal transfer mechanisms, and the influence of thyroid hormone thereupon, has been extended to include galactose. It is well known that the absorption of glucose and galactose by the intestine involves an active transfer mechanism;[6] it is likewise known that such a mechanism is involved in the renal tubular absorption of glucose.[7] The behavior of the renal tubules toward galactose is less clear.[8]

Experimental. I. The renal tubular absorption of galactose has been studied by the use of the method of Shannon and Fisher[9] in 2 female dogs in the normal state and in one animal after the administration of 9 daily doses (40 mg) of thyroxin. The details of our experimental procedure were as described previously,[4] with the exception that each urine collection period was extended to about 25 minutes (exactly timed). An interval of one-half hour was allowed for the establishment of equilibrium of plasma levels in those experiments involving changes in the concentration of galactose in the plasma. Reducing values were determined according to the method of Hagedorn and Jensen (1923). Filtrates (plasma and urine) were cleared of glucose by yeast fermentation. The non-sugar reducing substances were, in the case of urine, removed by precipitation with mercury[10] and, in the case of plasma, determined on suitably prepared blanks.

Results. It is evident from the data presented in Table I that within the limits of a four-fold change in the concentration of galactose in the plasma, a relatively constant fraction (about 40%) of the galactose filtered through the glomerulus is absorbed by the tubules. It is also apparent that within the limits studied (Creatinine U/P ratios, 8-67), the fraction absorbed is independent of the degree of concentration of the glomerular filtrate. The data for the animal in the hyperthyroid state suggests that thyroid hormone is without influence on the renal tubular transfer of galactose. It is unfortunate that circumstances prevented the accumulation of a greater amount of data on the animals in the hyperthyroid state. However, the data is more significant than at first appears, for the animals that were employed in this investigation were the same as those used in our previous study of the influence of thyroxin on the glucose Tm and the diodrast Tm.[4] Since these 2 studies were carried on simultaneously, and since Dog C showed a marked increase in the tubular transfer of glucose as a result of the administration of thyroxin only a few days prior to the galactose study, there can be little doubt that the galactose data was typical of the hyperthyroid animal.

Discussion. A comparison of the behavior

1 Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1938, **123**, 577.

2 Althausen, T. L., Lockhart, J. C., and Soley, M. H., *Am. J. Med. Sci.*, 1940, **199**, 342.

3 Eiler, John J., Stockholm, M., and Althausen, T. L., *J. Biol. Chem.*, 1940, **134**, 283.

4 Eiler, John J., Althausen, T. L., and Stockholm, Mabel, *Am. J. Physiol.*, 1944, **140**, 699.

5 Eiler, John J., Althausen, T. L., and Stockholm, M., to be published soon.

6 Verzar, F., and McDougall, E. J., *Absorption from the Intestine*, Longmans, Green & Co., 1936.

7 Smith, Homer W., *Lectures on the Kidney*, University Extension Division, University of Kansas, 1943.

8 Harding, V. J., and Grant, G. A., *J. Biol. Chem.*, 1933, **99**, 629.

9 Shannon, J. A., and Fisher, S., *Am. J. Physiol.*, 1938, **122**, 765.

10 West, E. S., and Peterson, V. L., *Biochem. J.*, 1932, **26**, 1720.

TABLE I.
Renal Tubular Absorption of Galactose.

			Creatinine			Galactose			
Dog	Date	State of animal	Plasma mg %	U/P ratio	Clearance cc/min	Plasma mg %	Filtered mg/min	Absorbed mg/min	Ratio Absorbed / Filtered
B	3/5/42	Normal	32.0	12.7	44.6	229	102	44	.43
			29.3	7.9	47.4	229	109	40	.37
			28.7	7.8	46.5	229	107	44	.41
	3/10		28.6	9.8	53.7	113	61	25	.41
			28.2	9.2	47.0	107	50	18	.36
			28.1	13.1	47.0	102	48	18	.38
			31.3	12.3	47.9	229	110	39	.36
			31.4	10.2	46.8	292	137	52	.38
								Avg	.39
C	7/7	"	31.7	12.8	67.6	179	122	70	.51
			27.8	23.1	64.7	175	113	71	.63
			26.5	40.4	68.6	165	113	66	.58
	7/9		32.6	9.8	62.0	371	230	92	.40
			28.2	16.5	66.0	415	274	120	.44
			27.8	13.6	65.5	446	292	116	.40
								Avg	.49
	5/29	Hyperthyroid	28.2	43.2	73.4	158	116	48	.41
			25.2	67.1	80.5	146	118	59	.50
			24.1	49.8	89.6	197	176	79	.45
			23.5	—	92.8	241	224	100	.45
								Avg	.45

of the intestine and the renal tubules toward glucose and galactose shows that while the intestine handles these 2 sugars in a similar fashion, there is a notable difference in the treatment they are accorded by the renal tubules. The question arises whether or not the differences in renal tubular absorption of these sugars is due to the absence of an active mechanism for the transfer of galactose. The present data is compatible with passive diffusion providing it is assumed, as has been shown to be the case for glucose, that the distal portions of the tubules are relatively, or actually, impermeable to galactose. The constant fraction of filtered galactose that is absorbed would then be occasioned by the diffusion gradient effected by the obligate[11] absorption of water in the proximal tubules, which in the range of U/P ratios studied (8-67) represents a constant fraction of the glomerular filtrate.[12] However, such an interpretation necessitates the conclusion that under a given diffusion gradient the membranes of the proximal tubules are equally permeable to galactose and urea, for Shannon[13] has estimated that about 40% of the filtered urea is absorbed by virtue of diffusion in the proximal tubules. Unless it be assumed that phlorhizin alters the permeability of the proximal tubular membranes toward monosaccharides, in addition to inhibiting a transfer mechanism, studies involving the use of this drug militate against a high rate for the penetration of sugars. Additional evidence may be cited against passive diffusion. A comparison of the data for galactose with that obtained from a study

[11] Smith, Homer W., *The Physiology of the Kidney*, Oxford University Press.

[12] Walker, Arthur M., and Bott, Phyllis A., *Am. J. Physiol.*, 1941, **134**, 580.

[13] Shannon, J. A., *Am. J. Physiol.*, 1938, **122**, 782.

of the tubular transfer of xylose[14] would indicate that the differences in the tubular absorption for glucose and galactose is not to be attributed to the absence of an active mechanism for the transfer of galactose. The tubular transfer of galactose and xylose is quite similar in several respects; the fraction of the filtered sugar that is absorbed is independent, in both cases, of the concentration of the sugar in the plasma, and of moderate changes in the degree of concentration of the glomerular filtrate. A demonstrated difference lies in the magnitude of the fraction absorbed. In contrast to 40% for galactose only 27% of the filtered xylose is absorbed. The observation that elevation of the plasma glucose level completely and reversibly blocks the tubular absorption of xylose, together with conclusions drawn from a sound, critical analysis of the kinetics of transfer mechanisms, has led Shannon[14] to conclude that xylose is absorbed by an active tubular process which is identical with that responsible for the absorption of glucose. Studies involving the use of phlorhizin would appear to support such a conclusion. Since galactose is absorbed by the renal tubules at a greater rate than xylose, it seems reasonable to conclude that the tubular absorption of the galactose also involves an active transfer mechanism. The difference in behavior of glucose and galactose, including the differences in their response to thyroxin, may then be attributed to a difference in the rate determining step in the sequence of reactions responsible for the active transfer.

Summary. The renal tubules of the dog absorb about 40% of the galactose that appears in the glomerular filtrate; within rather wide limits the fraction that is absorbed is independent of the concentration of plasma galactose and of the degree of concentration of the glomerular filtrate. In contrast to glucose, the renal tubular transfer of galactose is not altered by the administration of thyroxin. It is likely that a transfer mechanism is involved in the tubular absorption of this sugar.

[14] Shannon, J. A., *Am. J. Physiol.*, 1938, **122**, 755.

14597 P

Electrophoresis of Purified Prothrombin.

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Prothrombin has not been identified as a separate component in the electrophoresis patterns of oxalated or citrated plasma, because it is not present in sufficient concentration to permit detection. For this reason extensive electrophoretic data can be obtained only on concentrated preparations of prothrombin. In previous purification work[1,2] impurities have been removed in progressively greater proportion. In recent work we have obtained preparations of still higher purity. This offered an opportunity to study the electrophoretic properties of prothrombin for the first time.

The Tiselius apparatus used for this work is equipped with a Schlieren type lens and a Svensson diaphragm. The mobilities were calculated from observations of the descending boundary, in accordance with the recommendations of Longsworth and MacInnes.[3] The values for any given pH represent the average of several determinations. A new prothrombin preparation was made for each run. It is planned to offer a comprehensive presentation

[1] Seegers, W. H., Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Biol. Chem.*, 1938, **123**, 751.

[2] Seegers, W. H., *J. Biol. Chem.*, 1940, **136**, 103.

[3] Longsworth, L. G., and MacInnes, D. A., *J. Am. Chem. Soc.*, 1940, **62**, 705.

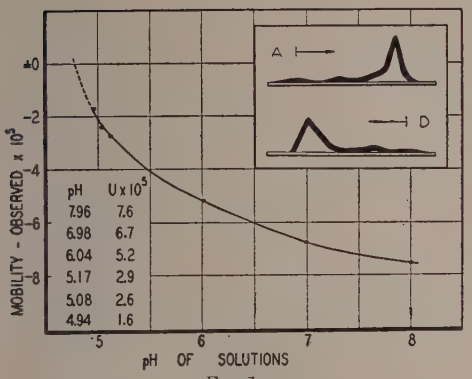

FIG. 1.

of the purification procedures at a later date.

A typical pattern, reproduced in Fig. 1, shows that the prothrombin component represents 80 to 90% of the total proteins in this preparation. The latter possessed 12,000 units[4] of prothrombin per mg tyrosine. By calculation pure prothrombin can be expected to have from 13,000 to 15,000 units activity per mg tyrosine or roughly 1,300 to 1,500 units per mg dry weight.

With similar preparations the mobility was studied by varying the pH. The curve obtained is given in Fig. 1. These results were obtained with numerous different preparations, dissolved in veronal, acetate, or phosphate buffers of 0.1 ionic strength. In the region of pH 6, 7, and 8 the mobility of prothrombin is greater than that reported or obtained by ourselves for the more concentrated plasma protein constituents such as albumin, fibrinogen, or the α, β, and γ globulins.

Since prothrombin is denatured in the region of or below pH 4.9, it was impossible to determine the isoelectric point accurately. By extrapolation it seems likely that it is pH 4.8 or less. It is obviously not pH 5.3 as might have been expected from the fact that prothrombin can be precipitated from diluted bovine plasma at pH 5.3. It is of interest to recall that acids inactivate prothrombin at pH 4.8.[2] Consequently it is almost certain that the isoelectric point and inactivation point are identical.

It has been shown that the conversion of prothrombin to thrombin can be blocked by heparin and an unidentified plasma factor.[5] Moreover, heparin alters the electrophoretic properties of certain plasma constituents.[6] It seemed desirable, therefore, to determine whether heparin alters the electrophoretic properties of prothrombin. The addition of heparin, in 0.4% concentration, produced no change in the mobility at pH 7.0.

Summary. The electrophoretic mobility of bovine prothrombin is greater than that of the more concentrated plasma protein constituents. At pH 7, heparin does not alter the mobility of prothrombin. The isoelectric point of prothrombin is in the region of pH 4.8 and in all probability is identical with the inactivation point. It is estimated that pure prothrombin will possess 1,300 to 1,500 units activity per mg of protein.

We wish to thank Miss Ann Garrett for technical assistance.

[4] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[5] Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

[6] Chargaff, E., Ziff, M., and Moore, D. H., *J. Biol. Chem.*, 1941, **139**, 383.

14598

Oxidized Cellulose and Thrombin.

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The use of absorbable cellulose[1,2,3] as a vehicle for thrombin to control hemorrhage offers new possibilities for securing effective hemostatis.[4]

We have observed that some combinations of thrombin and oxidized cellulose are damaging to thrombic activity. Further investigation has shown that there are two factors which can cause the inactivation. One is the inherent acidity of the oxidized cellulose, and the other is an unidentified product of the oxidation process. In order to demonstrate these facts it is necessary to vary the oxidation of the cellulose, in a manner which will separate the two factors which contribute to thrombin denaturation.

The oxidation of cotton with nitrogen dioxide gas has been described by Yackel and Kenyon,[1] and by Unruh and Kenyon.[2] These authors have also shown that the carboxyl content of the material increases as oxidation progresses. The carboxyl content can be measured by a simple titration, and by that means the progress of oxidation can be followed. We were supplied with oxidized cotton from the laboratory of the authors mentioned above, and had an opportunity to study its effect on thrombin.

Material with a carboxyl content of 14% was first studied. Approximately 100 mg of the cotton was mixed with 5 cc of bovine thrombin prepared by the method of Seegers.[5] The pH of the solution, measured with a glass electrode, was found to be below 4.3, which is the inactivation point for thrombin. With the use of standardized fibrinogen[6] the thrombin activity of the solution was quickly measured several times during the first 15 minutes after mixing, and was found to be disappearing very rapidly (Fig. 1).

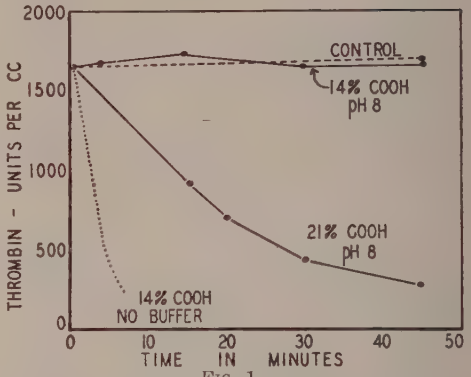

FIG. 1.

Mixtures of oxidized cellulose and thrombin are shown to be stable at pH 8 provided oxidation of the cellulose is not excessive. Without buffers the rate of thrombin inactivation varies markedly with the relative quantity of cellulose mixed with the thrombin.

In order to show that such loss of activity is due only to the acidity of the cotton, the same experiment was repeated with only one change in technic. The cotton was first soaked in 10 cc of 1% $NaHCO_3$ solution for 3 minutes, roughly dried between filter papers, and mixed with 5 cc of thrombin solution. The latter remained alkaline (pH, 8) and retained its original activity in full.

When oxidation of the cellulose is more extensive than required for rapid *in vivo* absorption by tissues, acidity continues to be a factor, but in addition other products of oxidation destroy thrombin. The inactivation process is slow, but eventually all active protein is destroyed. Fig. 1 shows the decline in activity, expressed in quantitative units,[6] when the carboxyl content is 21%. The cotton (100 mg) was soaked in 10 cc of 1% $NaHCO_3$ solution for 3 minutes, and then blotted dry between filter papers. When this

1 Yackel, E. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, 1942, **64**, 121.

2 Unruh, C. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, 1942, **64**, 127.

3 Frantz, V. K., *Ann. Surg.*, 1943, **118**, 116.

4 Putnam, T. J., *Ann. Surg.*, 1943, **118**, 127.

5 Seegers, W. H., *J. Biol. Chem.*, 1940, **136**, 103.

6 Seegers, W. H., and Smith, H. P., *Am. J. Physiol.*, 1942, **137**, 348.

bicarbonate treated material was placed in 5 cc of thrombin solution the resulting pH was 8; but the thrombin activity disappeared even though the acidity had been controlled properly. The nature of the destructive factor(s) is not known.

Summary. Oxidized cellulose injures thrombin due to its acidity. After neutralization with sodium bicarbonate it can be mixed with thrombin without altering activity. Highly oxidized cellulose contains products capable of destroying thrombin even in neutral solutions.

14599

Influence of Anesthesia on Circulatory Changes in Dogs Subjected to Graded Hemorrhage.*

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One of the chief difficulties in a study of the shock syndrome in dogs has been the lack of a critical evaluation of the effects introduced by the anesthetic agent. This paper deals with an analysis of the effects of several anesthetic procedures on the circulatory changes in the dog resulting from a standardized bleeding procedure. By using the changes in the omental circulation as an index it was found that criteria, previously established,[1,2] served as a means of differentiating between specific effects of different anesthetic agents.

Method. Fifty-three dogs were anesthetized with the 6 different drugs indicated in Table I: (1) procaine—local anesthesia, (2) morphine sulphate, (3) cyclopropane, (4) ether, (5) sodium pentobarbital, (6) sodium pentothal. Before anesthesia was induced the femoral artery was cannulated during local procaine infiltration (2 cc of 1% solution). Blood pressure were recorded continuously. Following induction of anesthesia the omentum was exteriorized for direct microscopic study according to a method previously described.[3] In Group 1, (Table I), which served as controls, pain relief for the required surgery was obtained with an abdominal field block using 8-10 cc of a 1% procaine solution. Group 2 was sedated with morphine sulphate given intravenously (2 mg/kg). Groups 3 and 4 were anesthetized with cyclopropane and ether respectively, administered with oxygen. The endotracheal to and fro absorption technic was employed. They were maintained unconscious, in upper first plane surgical anesthesia throughout.[4] Group 5 received a single intravenous dose of 25-30 mg/kg of sodium pentobarbital. Oxygen by endotracheal catheter was given to 3 dogs in this group. As no demonstrable differences were noted in the dogs with or without oxygen in this group, the results are considered together. Group 6 received sodium pentothal (1% solution) intermittently by vein to maintain a similar degree of narcosis and required an average of 10.8 mg/kg/hr. All of this group were given oxygen. Hemorrhage in each instance was accomplished with an initial blood-loss of 2% body weight and continued by removing an additional 0.5% at successive 30-minute intervals. To standardize the procedure, the maximal blood-loss compatible with viability was considered to be that which brought about a cessation of blood-flow in the larger omental arteries under observation during the bleeding. The majority of the dogs lived for at least 60

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1 Zweifach, B. W., Lee, R. E., Hyman, C., and Chambers, R., *Ann. Surg.*, in press.

2 Zweifach, B. W., Lowenstein, B. E., and Chambers, R., *A. J. P.*, in press.

3 Chambers, R., and Zweifach, B. W., *Am. J. Anat.*, in press.

4 Guedel, A. E., *Inhalation Anesthesia*, Macmillan and Company, 1937.

TABLE I.
Data on Dogs Subjected to Hemorrhage During Various Anesthetic Procedures.

Anesthesia	No. of dogs	Normal mean B.P.	Hematocrit (%)	Plasma protein (g %)	Anesthetic dose	Oxygen	Blood-loss (% body wt)
Procaine (Abdominal Block)	13	147 mm	50 ± 3	6.0 ± 0.5	8-10 cc	none	5.30
Morphine Sulphate	10	142 mm	45 ± 3	5.8 ± 0.5	2 mg/kg	none	4.60
Cyclopropane	9	152 mm	43 ± 6	5.55 ± 0.5	Upper 1st plane Surgical Anes.	used	4.78
Ether	5	139 mm	49 ± 2	5.67 ± 0.3	Upper 1st plane Surgical Anes.	used	4.10
Pentobarbital	10	147 mm	44 ± 1	5.80 ± 0.4	25-30 mg/kg	used in 3	3.70
Pentothal	6	164 mm	48 ± 3	5.76 ± 0.4	10.8 mg/kg/hr	used	3.80

TABLE II.
% Drop in B.P. from Initial Level at Various Degrees of Blood-Loss.

% Blood-Loss	0	2	2.5	3	3.5	4	4.5	5	5.5	Critical B.P. level mm	Status following last bleeding
Abdominal Field Block	0	17	—	35	37	51	59	62	62	35-45	Maintained for > 2 hr.
Morphine	0	16	—	25	32	39	46	51	45	40-50	Fell to extreme hypotensive levels in 90 min.
Cyclopropane	0	25	58	37	44	48	56	63	69	35-45	Maintained, tended to rise
Ether	0	16	28	36	52	42	50			75-85	Fell to extreme hypotensive levels in 42 min.
Pentobarbital	0	19	31	37	51	52	64			55-65	Fell to extreme hypotensive levels in 45 min.
Pentothal	0	29	49	44	45	45	40			80-90	Fell abruptly to extreme hypotensive levels in 63 min.

to 90 minutes after the final bleeding, those surviving for two hours, at which time observations were discontinued, being sacrificed. No sustaining infusions were used.

Results. Changes in the omental circulation were correlated with blood pressure changes at specific degrees of blood loss. The omental criteria studied were (a) the degree of curtailment of capillary blood flow, (b) the deviations in the minimal reactivity of the muscular components of the bed to the topical application of epinephrine, and (c) changes in the vasomotion of the central muscular channels. The term "vasomotion" is applied to the cyclic contraction-relaxation pattern which characterizes these vessels.[3]

Toleration of Blood-Loss. The ability of the dog to withstand hemorrhage varied significantly with the anesthetic agent used (Table I). Maximal blood-loss (5.3% of body weight) was obtained in the local anesthesia group. The dose of morphine used, although it produced only a euphoric state, significantly lowered the ability of the dogs to tolerate blood-loss. Of the 4 groups subjected to inhalation or intravenous anesthesia, those given cyclopropane were more nearly able to tolerate the maximal blood-loss, while those receiving barbiturates withstood the least hemorrhage.

Inadequacy of Blood Pressure as Criterion. The fall in blood pressure following equivalent bleedings varied considerably. No accurate correlation could be made between the blood pressure levels corresponding to equivalent blood-loss during anesthesia with any one agent or with different anesthetic procedures (Table II). The blood pressure served as a poor index of the dog's ability to withstand additional hemorrhage and was an unreliable prognostic indication of survival time.

In general (Table II), for each anesthetic group, different critical blood pressure levels were noted below which the animal's condition deteriorated rapidly. With pentothal and ether this critical level was reached with relatively high blood pressures (65-75 mm). With cyclopropane and local anesthesia it was not obtained until lower levels (35-45 mm) were reached.

BLOOD FLOW IN CAPILLARY BED

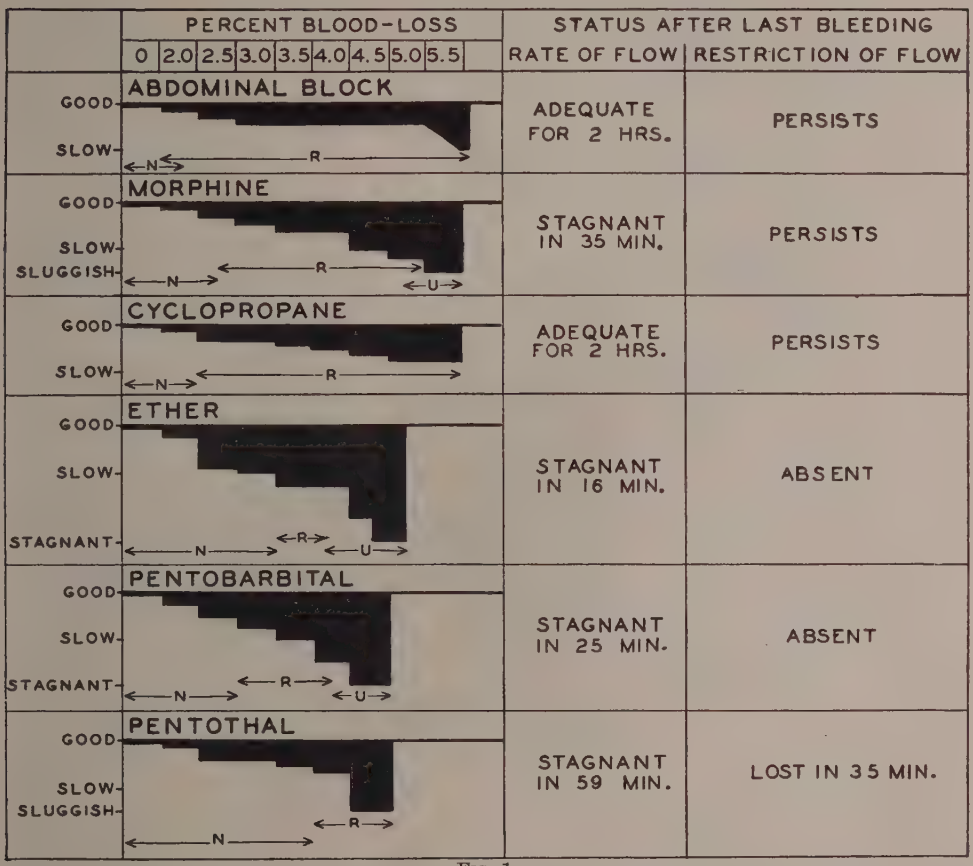

FIG. 1.

Changes in rate and distribution of capillary flow with respect to successive bleedings are shown; the degree of slowing by the deviation below the normal base line; the number of vessels open to the circulation by the symbols "N," "R," and "U." N = the normal condition, with an intermittent flow only through most capillaries. R= the restricted condition, with flow through most direct channels. U = unrestricted condition, with widespread flow through all capillaries.

Capillary Blood Flow. As noted in Fig. 1, the blood flow in the omentum varied considerably with the different anesthetic procedures. The feature which reflected most closely the ability of the dogs to withstand progressive hemorrhage was the persistence of a good flow in the capillary bed. This depended not only upon the rate of flow but on the number of capillaries containing an active circulation. As the hemorrhage approached maximal volume, the omental circulation slowed significantly in all groups (Fig. 1). In those groups which showed high blood-loss figures (control, cyclopropane, morphine), the omental blood flow remained adequate for the longest time. In those groups which showed low blood-loss figures, the last bleeding was followed by the development of a poor capillary circulation, with stagnation in many of the venules and backflow from the larger veins. Such a condition invariably indicated incipient circulatory failure. This occurred in the ether and pentothal groups despite the maintenance of relatively high blood pressures (75-90 mm).

In addition to a slowing of the blood flow,

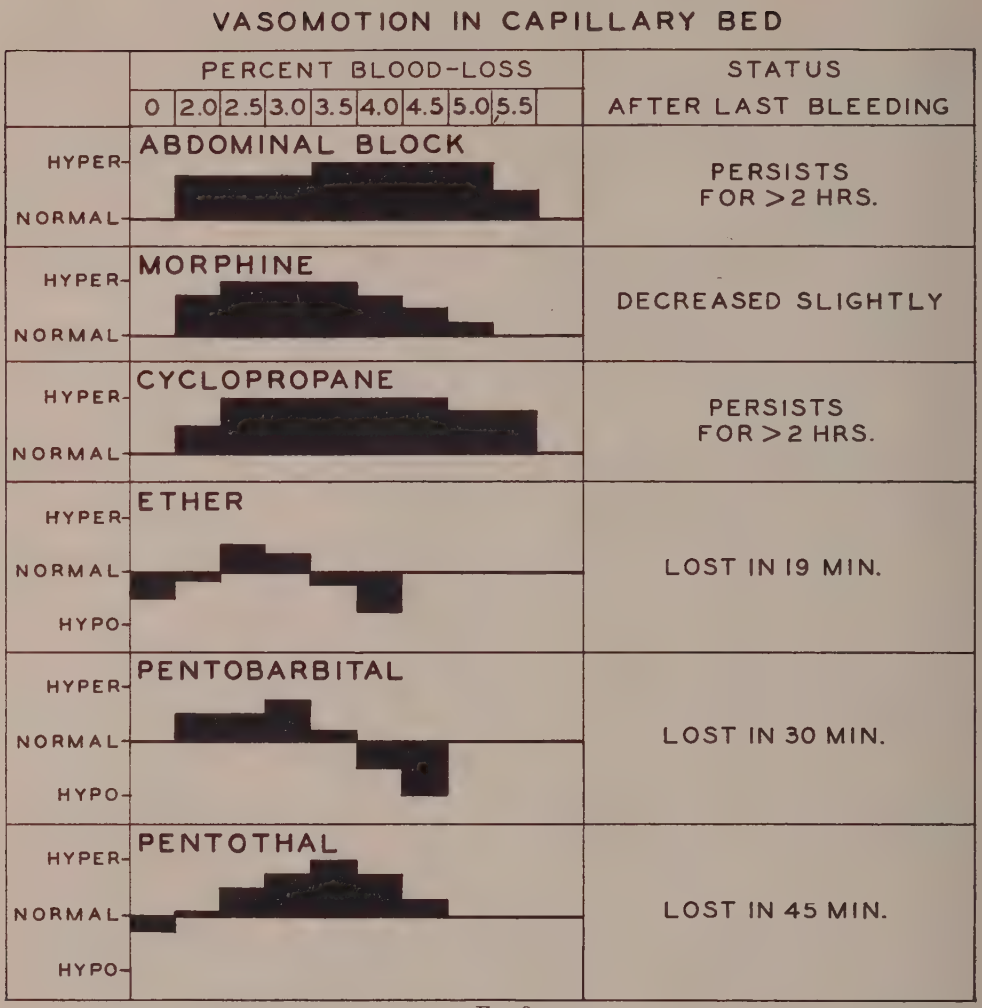

FIG. 2.

hemorrhage induced a compensatory restriction of the capillary circulation. This restriction appeared as an ischemic state in which the flow became confined to certain channels in the bed. The time of the appearance of this state and its duration are indicated in Fig. 1 by the symbol "R" below the graphs representing rate of blood flow. The loss of this compensatory reaction appeared as a state in which the blood flow, though deficient, spread throughout the entire capillary network. This unrestricted state is represented in the figure by the symbol "U." Loss of this compensatory mechanism was observed during ether, pentobarbital, and pentothal anesthesia and not with the other agents.

Vasomotion. As has been reported previously,[1] hemorrhage invariably results in the development of an increased vasomotion in the muscular channels of the capillary bed. The restriction of capillary flow was found to occur only during the period when the increased vasomotion was maintained. Loss of this activity was always accompanied by a filling of the entire capillary network and a marked decrease in the venular outflow.

Fig. 2 illustrates the changes in vasomotion following bleeding during the different anes-

TABLE III.
Epinephrine Reactivity of Omental Vessels Following Hemorrhage.

% Blood-Loss	0	2	2.5	3	3.5	4	4.5	5	5.5	Status after last bleeding
Abdominal Field Block	1x	2.2x	—	3.3x	4x	4x	5x	6x	10x	Persists high for >2 hr
Morphine	1x	1.5x	2.0x	2.2x	3.0x	3.5x	4.8x	5.8x	6.3x	Decreases only slightly in 90 min
Cyclopropane	1x	2x	2.5x	7.3x	10.6x	14.9x	14.2x	15.9x	13.3x	Persists high for >2 hr
Ether	1x	1.8x	2.1x	5.9x	5.5x	7.2x				Decreases markedly in 54 min
Pentobarbital	1x	1.6x	2x	2.2x	2.3x	2.5x				" " " 45 "
Pentothal	1x	2.5x	4.6x	5.6x	8.8x	9.3x	14.2x			" " " 73 "

Changes in epinephrine reactivity are indicated as multiples of the normal values prior to bleeding which are designated as 1x.

thetic procedures. Ether and pentothal, as compared with the control, dampened this activity even prior to hemorrhage. Following blood-loss during ether, pentobarbital, and pentothal anesthesia, the vasomotion progressively fell to hyponormal levels and was completely lost approximately 20 to 45 minutes after the final bleeding. In the controls and those receiving cyclopropane, a hypernormal condition was evident soon after the onset of hemorrhage and was maintained throughout the period of observation. Those receiving morphine resembled the control series except that the vasomotion slowed slightly during the final two bleedings.

Epinephrine Reactivity. The minimal effective concentration of epinephrine was determined for each dog prior to bleeding by applying the drug to the surface of the omentum and noting the concentration which produced a narrowing of the metarterioles[3] just sufficient to slow the capillary flow. In the controls, shortly after the initial bleeding there was a progressive increase in this reactivity (see Table III). With cyclopropane and pentothal anesthesia, the initial values, prior to hemorrhage, were higher than those of the control group. Despite this they showed the greatest increase in reactivity to epinephrine following hemorrhage. With pentothal, however, a marked decrease in this response occurred within 75 minutes after the final bleeding, in contrast to the cyclopropane group, in which this activity was maintained throughout. Those receiving ether or pentobarbital showed a rapid loss of post-hemorrhagic hyper-reactivity following the last bleedings. The dogs given morphine closely resembled the controls except that their response following bleeding increased only 6x as compared with 10x in the controls.

Summary and Conclusions. 1. Circulatory responses to graded hemorrhage varied significantly with six anesthetic procedures studied. 2. Of the drugs studied with the criteria used, the changes with cyclopropane most nearly approached those observed in animals receiving only local procaine. 3. The effects of anesthetic drugs must be taken into consideration in evaluating experimental shock studies.

INDEX

All preliminary manuscripts are indicated by the letter P after the number of the article